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(54) Title: USE OF AROMATIC ALDEHYDES AS INSECTICIDES AND FOR KILLING ARACHNIDS

(57) Abstract

Methods and compositions based upon natural aromatic compounds are provided, which find use as pesticides. The pesticides are formulated in a variety of ways, including dusts, sprays, shampoos, soaps and microcapsules, and can be bound to a solid support or provided as bait or directly impregnated into organic matter infested by or susceptible to infestation by a target pest. Pests controlled include aphids mosquitos, lice, ants, snails, slugs, cockroaches, lice, and ticks.

102(b) 37-39, 41, 42, 43, 45, 46, 47, 49, 51-54, 57, 58, 59
28, ~~29, 32~~

103(a) 44, 48, 50, 55, 56

Teaches a method of applying a composition comprising cinnamic acid ethyl esters to the skin of animals. Teaches that the composition can be prepared as an emulsion and applied as a spray or dip. Teaches the the composition can comprise an add'nal active ingredient besides the cinnamic acid ethyl ester. It is inherent that the method would treat the instant infestations

103(c) 28, 29, 32
↓
amts of ingredients (emulsifier and active)

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USE OF AROMATIC ALDEHYDES AS INSECTICIDES AND FOR KILLING ARACHNIDS

INTRODUCTION

Field of the Invention

The present invention is related to the biocontrol of insects and arachnids using compositions which contain aromatic compounds as a growth modulating agent. The method is exemplified by the biocontrol of aphids, two-spotted spider mites, flies, fleas, ticks, cockroaches, western subterranean termites, ants, mosquitoes, lice, biting midges, earwigs, slugs and snails with aromatic aldehydes, esters or acids.

Background

Organic matter, including decaying organic matter, is colonized by a variety of organisms, many of which are dependent upon a particular organic material as a source of nutrients. The colonizing organisms include a variety of insects and arachnids, some of which spread disease and/or damage the material which they colonize. The insects and arachnids which colonize particular organic materials include those species such as cockroaches, fleas, termites and spider mites which are symbiotic with bacteria; the host organism cannot survive without the symbionts. The colonizing organisms also include those which are disease vectors to mammals and include ticks, mites, fleas, and mosquitoes and various sap-sucking insects which are disease vectors to plants, and include aphids and thrips. The *arachnida* include sap-sucking plant parasites, the most important of which are the gall mites and spider mites which cause damage to agricultural and horticultural plants around the world.

Most orders of ticks include species of medical importance. Just the activity of blood-sucking ticks causes irritation and malaise in the host. However, the tick's role as carrier and transmitter of human disease organisms is of most concern medically. The organisms, chiefly viruses, rickettsiae and spirochaeta bacteria, are transmitted in the tick's saliva during feeding, and any one organism can be carried by a range of tick species. The viruses cause hemorrhagic fevers or encephalitis. The habitats of ticks include Canada, the U.S.A., Malaysia, India, and eastern, northern and central Europe. The different types of diseases caused by ticks usually are named after the place where they were first identified (e.g., Omsk hemorrhagic fever).

Another disease risk that is spreading geographically is Lyme disease (LD). LD is a multisystem inflammatory disease that in its early localized form affects the skin and joints,

nervous system and, to a lesser extent, other organic systems. Like a virus, rickettsia can develop only inside living cells. The main human rickettsial infections are the spotted fevers, tick-bite fevers and tick-typhus fevers, one of the most famous examples being Rocky Mountain spotted fever, which in the western U.S.A. is carried by the wood tick, Spirochaetes. The disease is characterized in humans by relapsing fevers and is transmitted by tick species of the genus *Ornithodoros*. These occur in Africa and the Americas.

In cattle, *Ornithodoros coriaceus* has been studied in order to gauge its relationship to bovine abortion. Epizootic Bovine Abortion (EBA) has become recognized as a major factor in preventing maximum range cattle calf production in California. Cows of various ages and breeds are susceptible to the disease, and abortion rates of up to 40 percent are not uncommon. *O. coriaceus* tested for vector ability were captured from EBA enzootic areas in California. After transport to the laboratory and acclimation, heifers were exposed to EBA by blood feeding. A cause and effect relationship between *O. coriaceus* blood feeding and subsequent disease was established. This soft tick disease represents a $30-$50 million problem in the state of California, with catastrophic loss years of approximately $100 million. Another disease vector affecting cattle is the soft tick which is the vector of numerous arboviruses.

Larval mites of the family *Trombiculidae,* commonly called chiggers or red bugs, are mostly lymph-feeding ectoparasites of vertebrates. About 20 species cause either a dermatitis (scrub-itch), resulting from an allergic reaction to the chigger's saliva, or transmit human disease organisms. Among the latter is the most important of mite-borne diseases, scrub-typhus or tsutsugamushi disease, which occurs in many parts of eastern and southeastern Asia. The best known mites which infect humans are scabies or itch mites. Scabies, known also to be a severe irritant to cattle, is highly contagious and its effects range from dermal irritation to death. Favored sites for infection are the hands and wrists; usually severe itching and rashes result.

House-dust mites induce allergic reactions in the form of asthma and rhinitis in humans. Several species of food mites cause a dermatitis in people handling infested food which include grocer's itch, associated with the presence of the flour mite. The crab louse, head *(Pediculus humans)* and pubic *(Phthirius pubic)*, also cause discomfort to humans. Lice act as vectors for exanthematous typhus, a disease caused by *Rickettsia prowazaki,* a rickettsia. Millions of deaths have resulted from this disease. In domestic animals, disease and, more importantly, weight loss due to irritation are caused by lice.

Mosquitoes, because of the pathogenic microorganisms they not only carry around but in some cases actively culture, are an important threat to human health. While particularly adept at

transmitting diseases caused by viruses, they also are known vectors of disease-causing nematodes and protozoans. The mosquito species probably the most closely associated with humans is that of the genus *Aedes*. There are about 150 species of this genus in North America; one, *Aedes vexans*, the inland floodwater mosquito, is known for its painful bite. In terms of human health problems, the most important species of *Aedes* is *A. aegypti*, which is the vector for an arbovirus that causes the disease yellow fever in humans.

Other arboviruses associated with the *Aedes* species include those which cause dengue fever; eastern and western encephalitis; Venezuelan equine encephalitis; St. Louis encephalitis; chikungunya; oroponehe and bunyamidera. Given this spectrum of disease, there is justifiable concern over the recent introduction (1985) of *A. albopictus* into the U.S. *A. albopictus* is a known vector of dengue fever and is a suspected vector of a number of forms of encephalitis, hemorrhagic fever and yellow fever. The genus *Culex* contains various species including the common house mosquito, *C. pipiens*. In North America, it is implicated in the transmission of various forms of encephalitis and the filarial worms *Wuchereria banufti* or *Brugia malayi* responsible for elephantiasis. Mosquitoes also may be the vector for Ebola, which is caused by a filovirus.

In the mosquito genus *Anopheles*, of which there are about 300 species worldwide, 15 species live in North America. While many species of mosquito feed on human blood, a majority of individual mosquitoes in the world do not; for them the consumption of human blood is distasteful and other vertebrate hosts are preferred, to which they spread disease. Certain *Anopheles* mosquitoes can act as vectors of pathogenic organisms that circulate in the bloodstream. Among these are protozoans in the genus *Plasmodium*, which cause the disease malaria in humans which afflicts between 200 and 300 million people and kills at least two million every year. Humans are affected by only four species of this genus: *P. vivax*, *P. ovale*, *P. malariae* and *P. folciparum*.

Other pests which can act as disease vectors include cockroaches. Cockroaches remain one of the most widespread and troublesome household and commercial pests, in spite of the rather extensive use of insecticides. The most pestiferous species of cockroaches in California is *Blattella germanic (L)*, the German cockroach. These cockroaches are found in grocery stores, restaurants, hospitals, jails, hotels, apartments, homes, in particular, in almost any place that food is stored. Most often they are associated with less than adequate sanitary conditions and are linked with the mechanical transmission of several pathogenic microorganisms. The droppings or skins of cockroaches cause hives or rashes, coughing, sneezing and contact or inhalant allergic

reactions in humans. Regular insecticide application is the usual means of cockroach control. The common strategy is to spray areas where the insect has been seen or is suspected to dwell. The ability of cockroaches to expand their populations rapidly, their close association with people and food, and their propensity to hide in inaccessible places make it difficult to exterminate them.

Formulations which are used for controlling insect and arachnid pests include the following: organophosphates such as malathion and ditrom; non-organophosphates such as pyrethrum and pyrethroids (synthetic pyrethrum); mineral oil; oil; methoprene; and bacillus thuriengiensis israelensis crystal protein. However, the wide-spread use of pesticides has resulted in the development and evolution of resistant pests, as well as growing environmental and health care concerns about the use of pesticides. As an example, the pesticide registration for malathion may be cancelled when it undergoes the reregistration process at the US EPA; the pesticide registration for DDT was similarly cancelled due to environmental and health care concerns. A highly visible ecological-environmental activist community and public regulatory agencies have resulted in fewer and fewer pesticide registrations in the United States and, consequently, less related research and development of pesticides. However, due to the dearth of effective pesticides, the toxic compound methyl bromide was recently reapproved for use on crops and in the rhizosphere in California. It therefore is of interest to identify and/or develop "biorational" formulations which have lower animal and environmental toxicities, yet are effective in controlling insect and arachnid pests.

Relevant literature

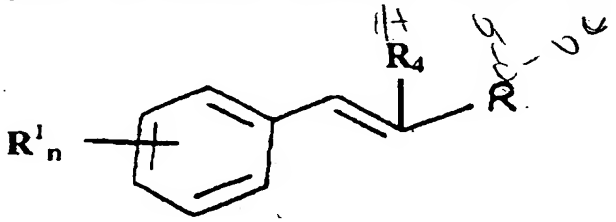
A method of protecting crops from attack of pests including insects using a composition comprising cinnamic aldehyde and requiring an antioxidant is disclosed in U.S. Patent No. 4,978,686. Protection of crops against insect pests by applying an aqueous composition containing a cinnamaldehyde is disclosed in French patent application 2529755. U.S. Patent No. 2,465,854 describes an insecticidal composition containing a cinnamic aldehyde derivative.

U.S. Patent No. 4,402,950 describes the deactivation of viruses inside living human and animal organisms by application of a terpene obtainable from aromatic plants by steam application. The terpenes cited are: black pepper oil, cinnamon flour oil, cardamon oil, linallyl acetate, cinnamic aldehyde, safrol, carvon and cis/trans citrao. Antifungal-antibacterial detergents containing cinnamic compounds are reported in U.S. Patent No. 4,477,361.

WO 96/20594 discloses a method for controlling an insect or an arachnid population. The method comprises contacting the insect or arachnid population with a formulation comprising 0.01 to 25 g/l of one or more compound of an aromatic aldehyde. The aromatic aldehyde includes cinnamic aldehyde, coniferyl aldehyde and α-hexyl cinnamic aldehyde.

SUMMARY OF THE INVENTION

The present invention provides a method for controlling insect and arachnid pest populations through nutritional mediation using compositions containing aromatic compounds. The method includes the step of contacting a target pest with an amount of an aromatic composition sufficient to control growth of the target pest. The composition can be provided in a variety of formulations. It also can be provided for target pests as a component of a trap. Optionally, the trap contains a chemoattractant for the target pest. The growth modulating agent generally is an aromatic ester, aldehyde or acid. When the habitat of the pest or arachnid is a host plant tissue or part, the growth modulating agent also is used to increase or induce resistance in the plant host to the insect or arachnid pest. The growth modulating agent increases accumulation of an aromatic aldehyde or increases cinnamic acid in the plant thereby impairing the growth and/or viability of an insect or an arachnid population which infests a surface and/or a part of the plant. Of particular interest are compounds of formula (1), below.



(1)

wherein R represents -CHO, -CH_2OH, -COOH, -$COOR_5$; n is an integer from 0 to 3; and each R^1 independently represents -OH, or an organic substituent containing from 1 to 10 carbon atoms and from 1 to 5 heteroatoms, wherein the total number of carbon and heteroatoms in all R^1 substituents of said compound is no more than 15; R_4 represents hydrogen or an organic constituent containing from 1 to 10 carbon atoms and R_5 represents an organic substituent containing from 1 to 10 carbon atoms and from 1 to 5 heteroatoms. These compounds include naturally occurring compounds, such as cinnamic aldehyde, coniferyl aldehyde, cinnamic acid, cinnamic ester and closely related compounds. Also of interest are alpha substituted aldehydes, such as alpha hexyl cinnamic aldehyde (HCA). The invention finds use in controlling pest

populations in areas of infestation, or areas susceptible to infestation and/or killing target pest populations.

DESCRIPTION OF THE FIGURES

Figure 1 shows the results of a bioassay of different concentrations of Storax against the melon aphid on chrysanthemum leaves.

Figure 2 shows the results of a bioassay of 0.6% Storax alone, 0.6% Storax plus 0.1% cinnamic aldehyde (CNMA), or α-hexyl cinnamic aldehyde (HCA) against the melon aphid on chrysanthemum leaves.

Figure 3 shows the percent mortality of melon aphid treated with α-hexyl cinnamic aldehyde in the indicated formulations.

BRIEF DESCRIPTION OF SPECIFIC EMBODIMENTS

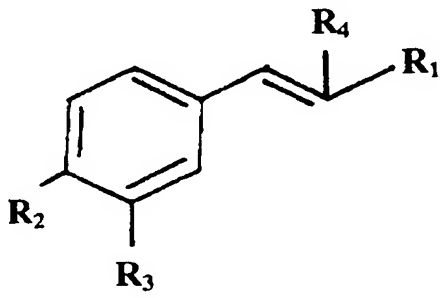
Methods and compositions are provided for obtaining and/or maintaining an area substantially free of pests such as insects and arachnids using compositions containing aromatic compounds to biocontrol the area. By "biocontrol" is intended control of pests via direct pesticidal activity on a target pest or by indirect pesticidal activity by antibacterial action on symbiont bacteria resident in the target pest. A target pest colonizing an area is contacted with a natural product. By "colonizing" is intended association of a pest with an area which provides access to organic matter which serves as a source of nutrients for the pest, typically essential nutrients such as amino acids, particularly methionine. By "natural product" is intended an organic compound of natural origin that is unique to one organism, or common to a small number of closely related organisms, and includes secondary metabolites provided by the organic matter. The natural products can be isolated from a natural source, be wholly or partially synthetic, or be produced by recombinant techniques. The amount of natural product that is provided, either applied to organic matter colonized by the target pest or as bait, will depend upon the degree of infestation of the area and to some extent upon the formulation and the specific compounding used and therefore should be empirically determined for particular applications.

The compositions and methods of the subject invention offer several advantages over existing compositions and methods, including that they are safe for use around humans, animals and food sources at the concentrations used. Additionally, the compositions can be used to impregnate organic matter which serves as a nutrient source for a target pest and/or can be

provided bound to a solid support which itself is non-toxic to animals, including humans. The formulation residuality also can be managed. This is of benefit when short term residuals are desired for integrated pest management programs with beneficial insects. In addition, the formulations are effective against pests which are resistant to other agents and also are effective on multiple target organisms, including insect targets known to be resistant to conventional treatments. This reduces the need for application of multiple agents for biocontrol of more than one target pest. Reentry time also is not an issue. Typically the formulations are rapidly lethal to a target organism; this is a particularly valuable characteristic when coupled with no reentry time. Another advantage is that the aromatic aldehydes in particular have positive organoleptic and olfactory properties which in some cases may improve the smell of treated area. The odor of HCA, for example, is described as floral or jasmine-like with some herbaceous character.

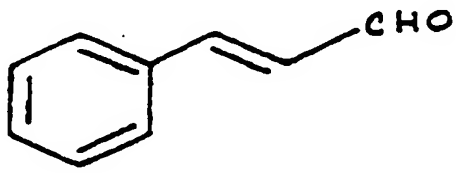
When applied to animals, including humans, the subject formulations are non-toxic and non-irritating to the skin at the concentrations used. For example, α-hexyl cinnemaldehyde (HCA) has an oral LD_{50} of 3.1 g/kg in rats and a dermal LD_{50} of greater than 3 g/kg (Moreno, O.M. Report to RIFM, March 24, 1971). HCA was found to be moderately irritating when the neat compound was applied to intact or abraded rabbit skin for 24 hours under occlusion (Moreno). When tested at 12% in petrolatum, HCA produced no irritation after a 48 hour closed-patch test on human subjects and produced no sensitization in a maximization test carried out on 25 human subjects (Kligman (1966) *J. Invest. Dermatol.* 47: 393). HCA at 20% in diethylphthalate produced no positive reactions in a repeated insult patch test conducted on 100 human subjects. In studies using the maximization test in guinea pigs, Senma and coworkers report a tendency that as the number of hydrocarbons of alkyl groups replacing the alpha-hydrogen in cinnamaldehyde increased, the rate of sensitization reaction declined.

The subject formulation is as shown in formula (1) above. A preferred formulation is shown in formula (2) below:



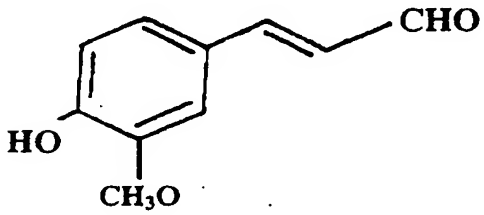
(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms. Of particular interest are aromatic aldehydes. Examples of aromatic aldehydes of use in the present invention are cinnamic aldehyde ((3) below):



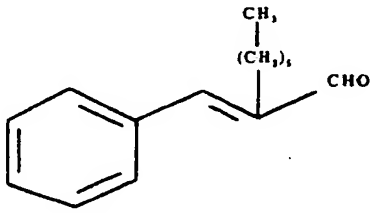
(3)

and coniferyl aldehyde ((4) below).



(4)

Other compounds of interest include analogs of the compound of formula (1) such as compounds substituted at the alpha position with an alkyl, such as a hexyl group, or a branched alkyl group such as an amyl group. Generally the group at the alpha position is from C-5 to C-10. Such compounds include alpha hexyl cinnamaldehyde and alpha amyl cinnamaldehyde. The chemical structure of alpha-hexylcinnamic aldehyde (HCA) is shown in (5) (below).



(5)

The Chemical Abstracts Service (CAS) name of HCA is 2-(phenylmethylene) octanal and the CAS Registry Number is [101-86-0]. The compound is also described by the chemical name of 2-hexyl-3-phenyl-2-propenal. The compound's formula is $C_{15}H_{20}O$ and molecular weight is

216.3. HCA can be obtained from Firmenich; their product is composed principally of the (E)-cis isomer (93.8% maximum), and the (Z)-trans isomer (6% maximum). Among minor components is the self aldol condensation product of octanal (1-1.5%) (Personal Communication, June Burkhardt, Firmenich, Plainsboro, New Jersey).

A number of the aromatic and aliphatic aldehydes which find use in the subject invention, such as benzaldehyde, acetaldehyde, cinnamaldehyde, piperonal, and vanillin are generally regarded as safe (GRAS) synthetic flavoring agents (21 CFR §172.515). Among these compounds is HCA. HCA was in public use before the 1950's and today is widely used in consumer preparations (soaps, detergents, creams, lotions, perfumes) (Monographs on fragrances raw materials. *Food Cosmet.Toxicol.* 12: suppl., 915, 1974). HCA was granted GRAS (generally recognized as safe) status by FEMA (Flavoring Extract Manufacturers' Association. Survey of flavoring ingredient usage levels. No. 2569. *Fd. Technol.*, Champaign, 19: (part 2) 155, 1965) in 1965 and is approved by the US FDA for use in food (21 CFR Section 121.1164). The Council of Europe (1970) (Council of Europe. Natural and Artificial Flavouring Substances. Partial Agreement in the Social and Public Health Field. Strasbourg, List A(1), Series 1, no. 129, p. 55, 1970) included HCA in the list of admissible artificial flavoring substances at a level of 1 ppm. In addition, surfactants which can be used as emulsifiers for the aromatic compounds. including the Tweens (polysorbates) already are used as food additives, as is saponin which also has GRAS status.

The aromatic and aliphatic aldehydes of the subject invention are prepared by various synthetic methods known to those skilled in the art. For example, *see*, J. March, ed., Appendix B, *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 2nd Ed., McGraw-Hill, New York, 1977. Cinnamaldehyde may be prepared synthetically, for example, by oxidation of cinnamyl alcohol (Traynelis *et al., J. Am. Chem. Soc.* (1964) 86:298) or by condensation of styrene with formylmethylaniline (Brit. patent 504,125). The subject aldehydes may also be obtained by isolation from natural sources. For example, cinnamaldehyde may be isolated from woodrotting fungus, *Stereum subpileatum*. Birkinshaw *et al., Biochem. J.* (1957) 66:188.

HCA can be synthesized as described, for example, in USPN 5,055,621. On a laboratory scale, HCA can be synthesized by reaction of benzaldehyde with octanal under a nitrogen atmosphere (aldol condensation). The reaction is conducted in a stirred flask charged with methanol, 309 ppm diphenylamine, potassium hydroxide and benzaldehyde. Following the slow addition of octanal. the reaction mixture is brought to a pH of 7.5-9.5 with acetic acid.

Following evaporation of methanol and wash of the reaction mixture with water, the organic phase is transferred to a distillation unit. Approximately 20-24% of the pot charge is removed as benzaldehyde and "lights", with the remaining distillate constituting alpha-hexylcinnamic aldehyde "heart cut." The "heart cut" is subjected to an additional fractionation, in which 1-5% (by weight) of the material may be removed in "light" fractions, depending upon odor evaluation. The final product is a light yellow oil having a specific gravity of 0.955-0.965 at 20°C, a refractive index of 1.548-1.562 at 20°C, a boiling point of 305°C at 1 atmosphere, and a melting point of 26°C. The commercial product is stabilized with the addition of 0.04% 2, 6-di-tert-butyl-*p*-cresol (butylated hydroxytoluene or BHT), which serves as an anti-oxidant (Technical Data Sheet, Hexylcinnamic aldehyde 907600, Revision 853, Firmenich Inc., Plainsboro, New Jersey). HCA also can be isolated from rice where it has been reported to occur naturally. (Givaudan-Roure Index, Givaudan-Roure Corporation, Clifton, New Jersey, 1994, p. 89).

HCA is a low to moderately volatile compound, having a vapor pressure of 70×10^{-5} mm Hg at 25°C. Its related compound, cinnamic aldehyde, has a vapor pressure approximately 40 times higher (2970×10^{-5} mm Hg at 25°C). For comparison purposes, the insect repellent N,N-diethyl-m-toluamine has a slightly higher vapor pressure (167×10^{-5} mm Hg at 25°C) (Reifenrath, W.G. (1995) *Volatile Substances. Cosmetics and Toiletries*, 110: 85-93).

An alternative to synthesizing aromatic compounds is to prepare them by recombinant means, for example, with a microbial host. The resulting microbes are used either to produce the aromatic aldehydes in a fermentation system or as a natural delivery system of the aromatic aldehydes in viable or non-viable microbial preparations. Yeasts, especially *Saachoromyces cerevisiae*, are preferred organisms for this purpose because they already have been engineered for high-level expression of phenylalanine ammonia lyase (Faulkener, J.D.B. *et al.*, Gene 143:13020, 1994) and a plant cinnamate 4-hydroxylase has been shown to function in yeast (Urban, et al. *1994 Eur. J. Biochem* 222:843-850.

The expression of phenylalanine ammonia lyase (PAL) in a recombinant organism provides the capability to the organism to produce cinnamic acid from phenylalanine. Two additional enzymatic steps are required to produce cinnamaldehyde from phenylalanine. In plants, these steps are catalyzed by the enzymes cinnamate CoA ligase (CL) and cinnamoyl CoA reductase (CCoAR) but as 4-coumarate CoA ligase (4CL) can also use cinnamic acid as substrate (Knobloch, and Hahlbrock 1977, *Arch. Biochem. Biophys.* 184:237-248), 4CL can be used instead of CL. More than 20 cloned PAL genes and more than 6 4CL genes have been described in sufficient detail (GenBank) to facilitate their use in practicing the current invention. A gene

for a CCoAR is obtained from plants by applying standard gene cloning techniques to isolate a cDNA clone using as a probe sequence derived from the amino acid sequence of the N-terminus, or peptide fragments, of the purified protein. CCoAR has been purified and partially characterized from soybean cultures (Wengenmayer *et al.*, (1976) *Eur. J. Biochem*, 65:529-536; Luderitz and Grisebach, *Eur. J.Biochem*, 119:115-124, 1981), spruce cambial sap (Luderitz and Grisebach, *supra*), poplar xylem (Sarni, *et al.*, *Eur. J. Biochem*, 139:259-265, 1984) and differentiating xylem of *Eucalyptus gunnii* (Goffner, *et al.*, *Plant Physiol.* 106:625-632, 1994). The preferred method of purification is that of Goffner *et al.* (*supra*) because it results in a single protein band on SDS-polyacrylamide gels that an be used for protein sequencing.

The cloned genes are introduced into standard expression vectors and used to transform a microbial host, preferably yeast, by standard transformation techniques such as electroporation (Becker and Guarante, *Methods in Enzymol*, 194:182-187, *1991*). Standard enzyme assays are used to confirm the functional expression of the engineered genes and assays for aromatic compounds are used to select strains with maximal production of a desired product. Because aromatic compounds have antimicrobial properties, it is preferred to use expression vectors that will cause expression of the introduced genes only late in the growth cycle or in response to a chemical inducer. It may also be desirable to grow the engineered microbial host in an immobilized whole cell reactor (*e.g.*, Evans, *et al.*, *Biotechnology and Bioengineering* 30:1067-1072, 1987) to prevent the aldehydes from accumulating in the culture medium.

In addition to the specific compounds of the formulas (1), (2), (3), (4) and (5) set forth above, derivatives of any of these compounds that produce a compound of the formula identified above upon action of a biological system on a precursor are considered to be equivalent to compounds of the invention. Thus application of precursor compounds to pests which can metabolize the precursors to produce a specific compound identified in the formulas above is equivalent to the practice of the present invention. Biological conversion of precursor compounds into aromatic aldehydes is described in, for example, U.S. Patent No. 5,149,715 and references cited therein. *See* also Casey and Dobb *Enzyme Microb. Techol.* (1992) 14: 739-747.

Additional components (other than those of formula (1)) can be added to the formulation to modulate the effect of at least one other compound present in the formulation whereby the combined action is greater than that without the addition of components and preferably is synergistic with the components of formula (1) in the formulation. By synergistic is intended that the activity of the formulation with the additional component as compared to a formulation which does not contain the component is greater than would be expected by adding the effects

together. An example of a synergistic compound is balsam (CAS number 8046-19-3) which finds use in the subject invention. A balsam is a resinous mixture of varying composition which is obtained from several species of evergreen trees or shrubs; it generally contains oleoresins, terpenes, and usually cinnamic and benzonic acids. Any of the balsams can be used, which include cinnamon compounds such as a cinnamic ester, phenopropyl cinnamate and free cinnamic acid. Of particular interest is Storax (also known as Styrax) obtained from the trunk of *Liquidamber Orientalis* Miller and American Storax from *Liquidamber sytraciflua*. The usual constitutents of Storax are 35-50% α- and β-storesin and its cinnamic ester; 5-10% styrene; 10% phenylpropyl cinnamate; small amounts of ethyl cinnamate; benzyl cinnamate; 5-15% free cinnamic acid; atyrene; 0.4% levoratatory oil; $C_{10}H_{16}0$, and traces of vanillin. The balsam can be combined with one or more aromatic aldehydes of formula (2), such as cinnamic aldehyde, coniferyl aldehyde and α-hexyl cinnamic aldehyde.

To obtain storax, the bark of an appropriate tree is bruised or punctured in the early summer, stimulating formation of balsam-secreting ducts. In autumn, the balsam saturated bark is peeled off and pressed. The residual bark is boiled in water and pressed again to obtain a second quantity of balsam. When *Liquidamber styraciflua* is the source of balsam, the exudate (Storax) collects in natural pockets between the wood and the bark and may be located by excrescenes on the trunk. Other balsams also can be used which produce a formulation having a desired antipathogenic and/or phytotoxic effect and are considered equivalents of the invention. Generally, an effective amount of storax when used in combination with an aldehyde such a cinnamic aldehyde (at 0.1%) or α-hexyl cinnamic aldehyde (at 0.1%) is 0.1% to 2%, is generally less than 1%, preferably 0.6% or less. The amount to use for various applications can readily be determined by comparing the effect with and without the balsam of compounds of formula (2) on a target organism.

Preferred additional components in the compositions include saponins. Saponins are a class of compounds, each consisting of a sapogenin portion and a sugar moiety. The sapogenin may be a steroid or a triterpene and the sugar moiety may be glucose, galactose, a pentose, or a methylpentose, for example. S. Budavari, ed., *The Merck Index*, 11th ed., Merck & Co., Inc., Rahway, N.J., 1990, p. 1328. Saponins for use in the present formulation include sterol glycosides widely distributed in plants, wherein each saponin consists of a sapogenin and at least one sugar moiety. The sapogenin comprises a steroid or a triterpene and the sugar moiety may comprise glucose, galactose, pentose, or methylpentose. The saponins for use in the present invention can be produced and/or isolated from various plant parts including fruit, leaf, seed

and/or root, using means known in the art, from a variety of sources including the various plants known to produce them, ranging from yucca, quillaja, agave, tobacco, licorice, soybean, ginseng and asparagus to aloe woods. Saponins have diverse activities which are attributable to the chemical make-up of a particular saponin and most typically are dependent on the source form which the saponin is derived. For example, saponins derived from Japanese Camilla control the growth of mosquito larvae. Saponins from sources other than Yucca plants can be used as active agents in insecticidal compositions.

Saponins for use in the present invention are preferably non-toxic to humans and higher animals. Most preferably the saponin for use in the present invention is a non-toxic food grade saponin, the source being, yucca plants with the most preferred saponins being derived from *Yucca schidigera* or *Y. valida* and their equivalents. Saponins from *Yucca schidigera* contain steroidal saponins with the major sapogenins being sarsapogenin and tigogenin. The sarsaponin yields on hydrolysis, sarsasapogenim (sarsasapogenim 5-beta, 20-betaF, 22-deltaF, 25-betaF; also known as spirostan-3-beta-01 and parigenin), glucose and galactose. The sarasapogenim has a molecular formula of $C_{27}H_{44}O_3$. Nobel, Park S., *Agaves*, Oxford Univ. Press, New York, 1994. Accordingly, derivatives of these compounds which produce a formulation having the desired pest growth controlling properties are considered equivalents of the invention. As appropriate, it is preferable to select a saponin that increases the pest growth controlling effect of a formulation as compared to a formulation that excludes the saponin.

The effect of saponin as an additional component in the formulation is determined by the addition of varying amounts of saponin admixed or applied separately in combination with a given formulation of aromatic compound(s). The effect of the formulation is measured by examining the susceptibility of particular pests to each formulation with or without a serial dilution of saponin. Generally, an effective amount of saponin is in the range of about 0.01 to 3% and most preferably about 0.25% v/v aqueous solution of 10° brix saponin extract. 10° brix is a term of art in sugar chemistry. The brix degrees equals the percent by weight of sugar in the solution. Hawley, ed., *The Condensed Chemical Dictionary*, 10th ed., Van Nostrand Reinhold, New York, 1981, p. 149.

Additional components such as an aqueous preparation of a salt of a polyprotic acid such as sodium bicarbonate, sodium sulfate, sodium phosphate or sodium biphosphate can be included in the formulation, where the addition increases the pesticidal properties of the formulation and/or confers other positive characteristics to the formulation, for example, by rendering it substantive for applications where it is desirable that a residue remain on the surface contacted

with the formulation. Generally, the formulations are effective without the use of antioxidants other than the inherent antioxidant properties of particular aldehydes, for example, coniferyl aldehyde.

Stability of the formulation can be evaluated by a variety of methods, including accelerated tests in which a formulation of interest is exposed to elevated temperatures over a set time. Samples of the formulations are taken at regular intervals and analyzed chemically by methods known to those skilled in the art to determine the rate and nature of degradation. For example, HCA can be analyzed by Gas Liquid Chromatography (GLC), using a 30 meter non-polar polydimethylsiloxane capillary column (e.g. HP-1, Hewlett-Packard, or SPB-1, Supelco) and a flame-ionization detector. Using helium as a carrier gas (8 ml/min.) and a column temperature of approximately 240°C, the (E)-cis isomer (major component) has a retention time of approximately 6.0 minutes and the (Z)-trans isomer (minor component) has a retention time of approximately 6.3 minutes.

Of particular interest is the addition of adjuvants to the formulation. By "adjuvant" is intended a substance added to a formulation to aid the operation of the main ingredient. A spray adjuvant performs this function in the application of an agricultural chemical. An effective spray adjuvant may be formulated to contain one or more surfactants, solvents or co-solvents. Systems containing surfactants, water and oily components have many other possibilities of forming ordered phases; the surfactant can organize itself into aggregates of various shapes to create micelles, with a first order phase as one of the possibilities. The surfactant also can collect at the interface between interpenetrating oil and water phases to create a microemulsion. Preferred surfactants for pesticides are the saponins. Saponins may be used both as an adjuvant and as a surfactant and also for reducing phytotoxicity when used in an agricultural application. For both phytotoxicity control as well as toxicological safety, preferred saponins are from *Yucca spp.*

The biocontrol compound(s) of formula (1) may be used either alone or in combination with other active or inactive substances and may be applied by spraying, pouring, dipping, in the form of concentrated liquids, solutions, suspensions, powders and the like, containing such concentration of the active compound as is more suited for a particular purpose at hand. The compound may be encapsulated in a polymer shell and applied in the form of microcapsules. In an application of controlling a plant pest, the shell material is preferably a biodegradable material. such as beeswax, carnauba wax, gelatin, sucrose, starch or dextran, so that the shell can be degraded to release the subject compounds to the target pest or its habitat. In the application of controlling an animal pest, the shell material is preferably an indigestible material, such as

beeswax, polyurea, polyamide, polyurethane, so that the microcapsules can pass through the digestive system of the host animal and will not stay in the host to cause any potential side effect. To encapsulate the subject compound in a polymer, a first prepolymer is dissolved in the core material of the subject compound. The resulting solution is then dispersed in the continuous phase (usually water), which usually contains one or more dispersing agents. A second prepolymer may then added to the resulting emulsion. A shell wall forming reaction occurs at the oil/water interface of the emulsion droplets. The resulting suspension of microcapsules which encapsulated the subject compound can then be further formulated to produce the final product. For example, cinnamic aldehyde (at 1 or 2%) and α-hexyl cinnamic aldehyde (at 0.1, 0.3, 1.0, or 5%) microencapsulated in a beeswax or carnauba wax solution at 1 micron size can be purchased from Sun Smart (Long Island, NJ).

The biocontrol compound(s) also may be applied, for example, in the form of dilute solution, in a suitable solvent such as water directly to an area of pest infestation or an area susceptible to infestation. When the subject compound is used as a means of cleansing a surface, such as a carpet, pet bedding, pet fur, clothing, skin, and the like, the aromatic compound can be formulated alone as an aqueous solution, it also can be prepared as a soap or a detergent. Detergents which can be used include anionic detergents such as those described in U.S. Patent No. 4,978,686.

At low concentration such as 10-500 ppm, the biocidal compounds of formula (1) can be used as attractions to attract insects or arachnids. For some applications the compound(s) are bound to a solid support for application in powder form or in a "trap". As an example, for applications where the formulation is to be used as a trap or as bait for a particular pest, the formulations of the subject invention can be sprayed directly in an area of infestation or they can be bound to a solid support or encapsulated in a time release material. Where a solid carrier is used, materials which can lead to oxidation of the active ingredients should be avoided. Examples of delivery systems include starch-dextran, and the like. *See* Yuan *et al.*, *Fundamental and Applied Toxicology* (1993) 20: 83-87 for examples of delivery systems.

The target pests include insects and arachnids, particularly those which colonize organic matter, more particularly those insects and arachnids that colonize organic matter which is an elicitor for the pest. By elicitor is intended that the organic matter provides nutrients required by the pest. Of interest as target pests, and the organic matter or habitat which provides their nutrients, are the following: Flies, (*Muscara domestica (L.)* and *Stomoxys calcitranus (L.)*, decaying organic matter, particularly matter which includes putrescine; fleas *Aphaniptera*

(Siphonaptera), blood ticks *Argas (Persicargas) arboreus (Ixodoidea:Argasidae)*, hard ticks (family *Ixodidae*), soft tick (family *Argasidae*), blood; *Dictyoptera: Blattellidae*, decaying organic matter; termites *Isoptera: Rhinotermitidae*, organic matter, particularly matter containing cellulose; ants (*formicidae*) including fire ants *Solenopsis invicta)*, carpenter ants (*Camponotus pennsylvanicus*), army ants *(Eciton)*; mosquitoes (*Aedes aegypti*), blood; lice (*Anoplura* and *Mallophaga*), blood. Also of interest is *Boophillus annulatus*, the hard tick associated with severe cattle problems in Australia and elsewhere and with mice, and lice Generally, lice are divided into two orders, the *Anoplura* (sucking lice) and the *Mallophaga* (all others, *e.g.*, elephant lice and chewing lice).

Also of interest as target pests are mites, such as spider mites (*arthropoda*), dust mites, mites which infect honey bees, and a variety of other mites, including those of the following orders: *Cryptostygmata* (beetlemite); *Mesostigmata* (red mite of poultry); *Prostigmata* (gall mite, water mite, chiggers and reg bug (follicle mite, quill mites); *Astigmata* (flour mite, furniture mite, fur mite, scabies or itch mite, fuschia mite and dust mite). It is a theory of the invention that many of the insects and arachnids which are susceptible to treatment with the subject formulations are those which harbor symbiotic bacteria in their gut. Accordingly, insects and arachnids other than those listed which harbor symbiotic organisms also can be controlled with the subject formulations.

In use, a formulation containing the pesticide is introduced to an area of infestation. For example, the formulation is sprayed on as a wet or dry formulation on the surface of organic material infested with a target pest, or organic material susceptible to infestation with a target pest. The formulation is usually air dried on the surface and the dry residues of the pesticide on the surface kill the target pest upon contacting of the pest with the surface. Alternatively, the formulation can be applied wet or dry to an area of infestation where it can contact the target pest. The formulation generally includes cinnamic aldehyde at 0.01 to 10%, preferably at 0.1 to 5%. Alternatively, the formulation includes α-hexyl cinnamic aldehyde at 0.5 to 50%, preferably at 1 to 20%.

In some instances, time-release formulations may find use, particularly for applications to animals, or areas which are subject to reinfestation, such as animal quarters. When used in a solid form or microencapsulated, the dosage used is typically on the order of 0.1% to 35% on a w/w basis, the maximum loading to be determined as a function of shell material selected. Analytical chemical techniques are used to determine and optimize rate of release. For qualitative purposes, GC techniques can be used to determine the amount of aldehyde released.

samples of encapsulated (pelletized) product are sampled at different time periods to measure release. Alternatively, volatile gases released from the formulation can also be analyzed. For measuring the activity of spray or powder applications, the stability of the formulations over time can also be evaluated by the GC methodology using techniques known to those skilled in the art. Methanol or alcohol extractions of the formulations also can be prepared and evaluated by HPLC analysis.

The aromatic compound(s) can be microencapsulated in a polymer and sprayed to plants susceptible for infestation by pests. For example, cinnamic aldehyde, coniferyl aldehyde or α-hexyl cinnamic aldehyde can be microencapsulated at about one micron size in beeswax or canauba wax. The encapsulated aromatic compounds are generally at a concentration about 0.1 to 20%, and preferably at 0.5 to 10%. The size of microcapsules useful for this invention is generally 0.1 to 50 micron, and preferably 1 to 20 micron.

The aromatic components can be coupled to a solid support, optionally through a linker such as a polysaccharidase binding domain, where the solid support is a polysaccharide such as cellulose, particularly microcrystalline cellulose. The preparation of cellulose binding domains is described in U.S. Patent Nos. 5,340,731; 5,202,247 and 5,166,317 and PCT application no. WO 94/24158. The aldehydes can be coupled to the binding domains, with or without a cleavable bond, using methods well known to those skilled in the art. These formulations can be used to directly impregnate a surface comprising the appropriate polysaccharide, for example where the surface is a cellulose, such as paper or wood, a cellulose binding domain is used. As an example, an aromatic aldehyde-cellulose binding domain composition can be used to impregnate wood which is subject to ifestation with or already infested with termites. In other applications, the aromatic aldehyde-cellulose binding domain composition can be bound to paper as a trap or to microcrystalline cellulose wherein the granules can be transported back to the colony. Optionally, the bait or trap additionally can include a chemoattractant for the target pest, such as putrescine for flies or cadaverine for cockroaches bound to the cellulose support via a cellulose binding domain. Other examples of insect and arachnid chemoattractants which are well known to those skilled in the art can be used with the subject aromatic compounds.

In addition to providing bait or traps, infestations of target pests also can be treated using powder or detergent formulations, for example as a carpet shampoo to treat infestations of dust mites and fleas and other susceptible pests. The formulations of the subject invention generally are non-staining and additionally often impart a pleasant odor to the treated surface. The formulations also can be used as emulsions or gels for treatment of infestations of animals or

humans, including infestations with fleas and ticks. Generally, the forn
ingestion at the concentrations used and additionally, typically have pc
olfactory characteristics.

In order to determine the susceptibility of particular pests to tl
vitro and in vivo tests such as are described in the Examples can be u
formulations also are evaluated for dermatological effects; where appropr
evaluation of the toxicity of the formulations is carried out on animal hosts for the target pest or
on animals which may come in contact with a treated surface so that the dermatological effects
can be determined for the dosage of pesticide used. Such dermatological sensitivity tests are
conducted using methods known to those skilled in the art. In some instances, it may be
necessary to adjust the treatment formulation so as to reduce any dermatological effects
associated with the formulation.

The method of the present invention is carried out by introducing into a target pest a sufficient amount of a pesticide to impair growth and/or viability of the target pest and thereby decrease the population of that pest in an area. The method of introduction of the subject pesticide into the target pest can be by direct ingestion by the target pest from a trap, or by feeding of a target pest on nutrient-providing organic matter treated with the pesticide. In some instances, the pesticide may be absorbed by the pest, particularly where the formulation provides for uptake by the outer tissues of the pest, particularly a larval or other pre-adult form of the pest, such as a detergent formulation. In some instances, the exoskeleton of the target pest is substantially dissolved by contact with the formulation. For some applications, it may be necessary to deliver the formulation to the location of the pest colony.

The method of use of the formulations will depend at least in part upon the pest to be treated and its feeding habits, as well as breeding and nesting habits. For example, a formulation including the aromatic compounds can be directly sprayed onto a target pest. Alternatively, the aromatic compound can be applied to an area of infestation where it can contact a target. The formulation generally contains 0.01 to 50% of the aromatic compounds, preferably 0.1 to 20%, and more preferably 0.5 to 10%.The following are examples of how to treat infestations of particular types of pests. For spider mites and relatives (as exemplified by the two spotted spider mite (*Tetranychus urticae*)), life stages include the egg, an early, six-legged immature stage, and eight-legged immature stage and the adult stage. With ambient and warm temperatures and low humidity, the generations are complete in as little as ten days. Adult females typically lay up to five eggs per day over the course of 14 to 21 days. The adult arachnid pierces plant cells and

feeds on the sap. There may appear small white flecking injuries surrounding the feeding mites, and generalized discoloration occurs, with bronzing as infestations progress. Vigor is reduced and premature leaf drop may occur. Raspberry, rose, bean, cucumber and marigold are among the most commonly and seriously damaged. Moreover, the two spotted spider mite is also the most common species that damages greenhouse crops and interim plants.

Spider mites are extremely difficult to control with pesticides, and many commonly used pesticides (e.g., Sevin) can increase problems by destroying natural predators. Miticides such as malathion and orthene are often ineffective because spider mites have developed resistance to them.

Ticks are the largest group of the subclass *Acari* and are obligate blood-sucking ectoparasites of land vertebrates. Certain species are pests of domestic livestock, while another group transmits human disease. Ticks are classified into three families, all but one species belonging to the *Ixodidae* (hard ticks) family or to the *Argasidae* (soft ticks) family. Hard ticks get their name from the thickened shield (scutum) on top of the front of the body. They possess prominent well developed mouthparts, which they use to secure themselves to their roving hosts during feeding, which can take several days. A common hard tick is the cosmopolitan brown dog tick. The compounds of the invention can be applied to the host as sprays, powder, dusts, shampoo and dips and can also be used to treat animal collars and/or bedding.

Soft ticks lack a scutum and have relatively weak mouthparts, positioned inconspicuously on the underside. Soft ticks are habitat ticks: they remain in the host's retreat and feed when it returns. Their mouthparts are not exceptionally well-developed, as the host is generally at rest while feeding proceeds. After feeding, soft ticks usually fall to the ground to lay eggs or molt. Compounds of the invention therefore can be used to treat nests and abodes, paddocks, pens, and the like, by spraying with an effective amount of the subject compounds.

Several species of ants (*Formicidae*) can be a nuisance in the garden, and inside the home, especially the kitchen area. Most species of ants in the United States are social insects that live in colonies or nests, in which remain the egg-laying queens, the young or larva, pupae and many worker ants. The workers, all sterile females, care for the colony as well as search for food and bring it to the nest. In the spring and fall, ant colonies may produce winged males which fly about, mate, and have the ability to start a new colony. Baits can be formulated which the ant will carry back to the nest.

Some species of ants construct mounds or small hills of granulated soil which may smother surrounding vegetation. Grass also may be killed as the soil around the grass roots dries

out from the effects of the digging and burrowing of the ants. Some species of ants which frequent turfgrass areas, and eventually construct anthills, include the little black ant *(Monomorium minimum)*, the pavement ant *(Tetramorium caespitum)*, and the thief ant *Solenopsis molesta)*. Compounds of the invention can be used to treat nests and anthills, as well as those areas in which they are likely to form, by treating with an effective amount of the subject formulation, either by adding formulations such as time release granules to areas of infestation and/or spraying the area with the subject formulation. Other ants may be in planted areas or near grass areas. The black carpenter ant *(Camponotus pennsylvanicus)* nests in dead trees, logs and even structural wood in houses. These large, winged, black ants often exceed 1 mm in length. Winged males and females may swarm occasionally. Baits and contact sprays can be used in eradication, and structural wood can be treated with the formulation, particularly with formulations prepared so as to bind to cellulose-containing materials.

The red imported fire ant *(Solenopsis invicta)* colony constructs honeycomb mounds containing up to 0.5×10^6 worker ants. These mounds are found in pastures, roadsides, field borders, and in home lawns. The ants build mounds in many areas but prefer sunny sites and clay soils over sandy soils. Fire ants increase their mound size in wet seasons to move above the moist areas. Soils used in nest and mound construction can be treated with a concentration of formulation to kill workers and soldiers and reduce mound size by spraying and/or applying release formulations for example, directly to the rhizosphere in areas of infestation.

Mosquitoes undergo a complete metamorphosis during their life cycle. Water breeding-eggs need H_2O to hatch (some species lay eggs on dry ground, others in water directly). Larvae are fast growing and shed skin four times in four to ten days. They feed on one-celled organisms and each other. Pupae do not eat and become adults in two to four days. Formulations of compounds of the invention can be used to treat environments that encourage accumulation of standing water (for example, stagnant ponds, discarded tires, pots, cans, and the like). In waterfowl areas (wetlands ponds, lakes, and the like), the concentration of formulations of compounds of interest can be adjusted to kill late stage larvae. Prolonging larvae life may provide waterfowl food since some species are reported to eat mosquito larvae (e.g., ducks). Adults can be controlled by spray contact insecticide containing an effective concentration of the subject compounds on surfaces including feathers, fur, clothing of target animals and birds, or to the insects in flight.

Slugs and snails are distributed throughout the world, frequently causing damage to glass-house and garden plants. Some slugs are especially injurious in mushroom houses. Young

seedlings and the more succulent parts of plants are devoured by these pests. They leave a trail of mucus on the surfaces on which they crawl, and, in drying, silvery marks appear; these are objectionable, especially on floral or ornamental plants. Direct contact spray when possible can be used to control the insects or spraying of traffic areas with an effective amount of the formulation. Alternatively, encapsulated formulations of the compounds of interest in a shell, particularly a chemoattractant shell can be placed in a trap or high traffic area.

Cockroaches undergo a gradual metamorphosis during their life cycle. Many oviparous-eggs are deposited with glandular secretions, harden to form a tough protective capsule-ootheca, which sticks to a substrate (usually concealed by debris) or carried on the end of the female's abdomen. The German cockroach is one of the most gregarious household pests and is distributed widely in the temperate regions of the world. This insect usually breeds and lives in buildings generation after generation without the influence of seasons. The presence of the German cockroach is undesirable. Food and dishes become contaminated by excreta and by various secretions. In recent years, an apparent change in its habits has been noted in areas where considerable amounts of insecticides have been used and where resistance of this species to chlorinated hydrocarbons and some organophosphates has occurred. Direct contact spray of nymphs and adults when possible can be used to control the insects or spraying of traffic areas (e.g., food prep areas, refuse areas, and the like) with an effective amount of the subject compositions. Alternatively, encapsulation of formulations of the compounds of interest in a chemoattractant shell can be placed in a trap or high traffic surface area.

Flies undergo a complete metamorphosis. Eggs are deposited in a moist habitat since the legless larvae require moisture. Parasitic flies are abundant in many environments and lay eggs in or on a vast range of animals, other insects, and vertebrates. Larvae are active predators of insects for flower visitors. Flies can be killed at the adult stage with an effective amount of the compounds of the subject invention formulated as a contact insecticide (for example, as a spray, a trap with sticky paper. other types of traps, and on solid bait).

Fleas undergo a complete metamorphosis. Larvae are free-living and legless with a developed head. Fleas are mammalian parasites and favor hosts that build nests, burrows, and dens. Larvae feed on host blood that has dried and passed out of adult flea as feces while host is in its lair. Larvae are vulnerable to climatic change they desiccate in dry conditions and can drown in a droplet of water. This vulnerability limits fleas to certain environments. Eggs, larvae and pupae (silk cocoon) develop freely in the nest or habitat of a host, e.g., feline and canine fur fleas are generally found to the hosts' nests (beds). Fleas shift from host to host and

feed indifferently on several kinds of animals. The cat flea (*Ctenocepalides felis* Bouché order Siphonaptera, family Pulicidae) is nearly as likely to be found on a dog or a human as on a cat. The subject compounds can be used to control fleas by contacting a host or its habitat with an effective amount of a formulation containing the subject compounds as a spray, dust or powder/ Alternatively, the subject compound can be encapsulated in an indigestible material such as beeswax suitable for passage through the digestive system of host mammals. The microcapsules will not stay in the mammals to cause potential toxic effects.

Termites undergo metamorphosis from eggs to larvae (nymph) to adults with no pupal stage. Nymphs may resemble the adult termite. Termites live in colonies for most of their life cycle stages. Termites can be treated with compounds of the subject invention by directly spraying an appropriate formulation on nymphs and adults. Wood surfaces with which termites come in contact also can be treated with an effective amount of a formulation. Termites can be brought into contact with microencapsulated formulations, particularly by binding the formulation to wood surfaces through a cellulose binding domain. Traps baited with pheromones and the compounds of the subject invention can also be used.

The cotton or melon aphid (*Aphis gossypii Glover*) fly to cotton plants almost as soon as cotton has put out leaves and start to reproduce. In a cool, wet season, when their natural enemies cannot work against them as well, they may become abundant enough to stunt and deform the plants which they have infected. Often, when hot weather or summer arrives, they practically disappear. A related aphid is the crown aphid which infests citrus plants. The subject compounds can be used to control aphids by contacting an aphid or its habitat with an effective amount of a formulation containing the subject compounds as a spray, dust, powder or encapsulated in a degradable material, which can be degraded to release the subject compounds to the target aphid.

Venomous spiders cause illnesses in mammals ranging from mild local inflammation to a severe systemic reaction. The most venomous spider in North America, the Black Widow *Latrodectus mactans* (Fabricius) and *L. geometricus* (Fabricius) are responsible for human mortality on the order of 0.2%. The other intensely venomous spider found in North America is the Brown Recluse spider *Laxosceles reclusa* (Gertsch and Malaik). Both males and females bite. Compounds of the invention can be used to treat nests and abodes and the like, by spraying with an effective amount of the subject formulation.

Scab mite (or Psoroptic Scab)(*Psoroptes equi* (Raispail) and *P. ovis* (Hering)) such as cattle scab mite is a minute whitish eight-legged mite that causes animal injuries by puncturing

the skin with its sharp mouth style. Cattle scabies is a quarantinable disease. Compounds of the invention can be used to control scab mites by contacting the host or its habitat with an effective amount of a formulation containing the subject compounds as a spray, dust or powder and the like.

The common bed bug (*Cimex lectularis*) and its close relatives (poultry bug (*Haematosiphon inodorus* (Duges), the European pigeon bug (*Cimex columbarius Jerjus*), and the swallow bug (*Oeciains vicarius Hrovath*), are frequently pests in poultry houses. At night the nymphs and adults find their way onto the sleeping hens and suck their blood. Sitting hens may suffer especially from these pests and may be driven to leave the nests. The bed bug will also attack humans, rabbits, guinea pigs, horses and cattle. On humans the bites become increasingly painful for a week or more. Bed bugs thrive under crowded and squalid conditions. The subject compounds can be used to bed bugs by contacting a host or its habitat with an effective amount of a formulation containing the subject compounds as a spray, dust, or powder, for example. The formulation can also be used to impregnate bedding and nesting materials; where these are cellulose-containing materials, the formulations can be bound to the cellulose via a cellulose binding protein.

Mealybugs, aside from strange appearance, are not too different than aphids, psyllids, and phylloxera. They suck the juices from plants and spread disease. The honeydew they excrete invites the growth of a sooty fungus which interferes with photosynthesis of the host plant. The compounds of the subject invention can be used to control mealybugs by contacting a mealy bug or its habitat with an effective amount of a formulation containing the subject compounds as a spray, dust, powder or encapsulated in a digestible material.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Materials and Methods

The chemicals used in the examples given below were obtained from the following sources: cinnamic aldehyde, Spectrum Chemical Company, N.J.; coniferyl aldehyde, APIN Chemical, U.K.; Tween 80 and sodium bicarbonate Spectrum Chemical Company, Gardena, California, alpha hexyl cinnamic aldehyde, Firmenich Chemical Manufacturing Center, Port Newark, New Jersey. Concentrations are given as the concentration of the indicated solution before dilution.

Example 1

Effect of Formulation on Spider Mite

Activity of cinnamic aldehyde and/or coniferyl aldehyde against two-spotted spider mite, *Tetranychus urticae* was determined as follows. In a double blind experiment, innersurfaces of petri dishes (60 mm diameter) were treated with 100 µl of a test formulation and allowed to air dry and used within the hour. Twenty adult spider mites were put in each dish and the percent of mortality of the spider mites after 24 hours in contact with the treated dishes was determined.

Table 1
Spider Mite

Formulation[1]	Percent Mortality
CNMA ppm	(24 hours)
25,000	99.2
12,500	98.6
5,000	66.4
2,500	78.0
100	56.0
10	51.7
Control	
HPLC H_2O	16.2
Vehicle[2]	49
Positive Control[3]	100
Neg. Control (H_2O)	12.6

[1]Cinnamic aldehyde at the indicated concentration in 2% Tween 80, 6% $NaHCO_3$.
[2]2% Tween 80, 6% $NaHCO_3$.
[3]Sevin 10 ppm.

After 24 hours in contact with a treated plate, the percent mortality data obtained with the various formulations was compared to that of spider mites in petri dishes treated only with water. Concentrations of cinnamic aldehyde ≥ 2500 ppm were more effective than vehicle alone, and produced ≥70% mortality.

Plant Foliar Bioassay

Cotton plants are grown in 7.5 mm pot in potting soil in greenhouse. When plants reach 3 leaf stage, they are infested with 60 adult spider mites (6 replications). The mite is allowed to settle and feed. The plant is sprayed to runoff (about 5 ml) with a formulation containing 100 to

2000 ppm, (0.1 to 2 g/l) concentration of a test formulation. The plant is covered with a tall plastic cage (5 mm tall x 10 mm diameter). The mortality of the spider mites on the plants sprayed with a test formulation is determined and compared with that of spider mites on plants sprayed with water only.

Example 2

Effect of Formulation on Flies

In an air conditioned case measuring 1.5 m x 1.5 m x 1.5 m, 150 flies (*Musca domestica Linnaeus*) and *Stomoxys calcitranus Linnaeus* are released and sprayed with 8 ml of test product. The test product contains 100 to 2000 ppm of cinnamic aldehyde and/or coniferyl aldehyde in an appropriate formulation. After 15 minutes exposure, the number of flies that are unable to fly are noted. All flies are transferred to a holding case with fresh air and allowed to recuperate for 20 hours. The number of dead flies are counted, and the percentage of flies killed with each formulation compared to that of no treatment and treatment with a formulation known to kill files at a level of about 70%.

Example 3

Effect of Formulation on Fleas

Petri Dish Bioassay

Aphanptera (Siphonaptera) susceptibility is tested as follows. Petri dishes (60 mm diameter) are treated with a specific dose of product (100 to 2000 ppm) dissolved with water, and allowed to dry. Twenty specimens of the insect and twenty larvae of the insect each are put in separate dishes (replicate 10 times). The mortality of insect and larvae after thirty hours in contact with a treated plate is compared to that of insects and larvae treated only with the diluent, and treatment with a formulation known to kill fleas at a level of about 70%.

Contact treatment

The treatment of cat flea (*Ctenocepalides felis*) with various formulations containing alpha hexyl cinnamaldehyde was tested as follows. In a double blind experiment, variable concentrations of the formulae were tested for activity against cat flea (*Ctenocepalides felis*). The initial experiments tested alpha hexyl cinnamaldehyde at concentrations of 5%, 10% and 20% in 6% Tween 80. As controls, a formula blank containing 6% Tween 80 was tested. Fleas were put in direct contact with the formulae and mortality was assessed both visually and by probing at 72 hours after contact.

Approximately 11,356 ml of each formula concentration was sprayed on a 0.279 square meter carpet section (DuPont) at 20 PSI. After allowing to air dry (20 minutes), four plugs each 14 cm in diameter were cut from each treated carpet section. One plug was used for each replicate for four total replications. For each treatment and replicate, 25 fleas were introduced on each plug. Plugs were then rolled and put in an escape proof, ventilated 2 liter container. After 72 hours mortality of the fleas was assessed.

All treatments using concentrations of alpha hexyl cinnamaldehyde yielded greater than 80% flea mortality. Fourteen percent mortality was observed with the formula blank of 6% Tween 80. See Table 2.

Table 2

Cat Flea

Formulation	Percent Mortality
HCA (%)*	
5	84
10	92
20	86
Control	
6% Tween 80	14
No spray	3

*HCA = alpha hexyl cinnamaldehyde (% wt/vol) in a vehicle of 6% Tween 80.

Example 4

Effect of Formulation on Ticks

In a double blind experiment, filter papers (90 mm) (Whatman) were treated to uniform saturation with 1 ml of test formula, air dried and placed in 90 mm petri dishes. Ten arachnids were placed in each petri dish to contact the filter paper and the dish closed. Observations of mortality were made at 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours and 24 hours. The cinnamic aldehyde concentrations varied from 10-50,000 ppm in a vehicle of 2% Tween 80, 6% $NaHCO_3$. The effect of vehicle alone or H_2O (HPLC) was also tested. In separate experiments, the effects of the components of the vehicle were evaluated in comparison to water.

In preliminary experiments with the hard ticks *(Ixodea pacificus* and *Dermacentor albipietus),* 100% mortality was achieved in 24 hours, at a concentration of 2500 ppm in vehicle. At concentrations below 2500 in vehicle, there was no effect on mortality. No effect was observed with H_2O or vehicle alone.

In preliminary experiments with the soft tick *(Ornithodoros coriaceus),* 100% mortality was achieved at concentrations at or above 12,500 ppm in vehicle (Trial 2); and 70% at a concentration of 12,500 ppm in vehicle (Trial 1). No effect was observed with H_2O or vehicle alone. See Table 3.

Table 3

Ornithodoros Coriaceus

(Soft Ticks)

	Number Dead		
Formulation[1]	Trial 1 24 Hours (#/30)	Trial 1 120 Hours (#/30)	Trial 2 120 Hours (#/10)
CNMA (ppm)			
50,000	NT[3]	NT	10/10
45,000	NT	NT	10/10
40,000	NT	NT	10/10
35,000	NT	NT	10/10
30,000	NT	NT	10/10
25,000	7/30	21/30	10/10
12,500	5/30	6/30	10/10
5,000	3/30	6/30	NT
2,500	2/30	6/30	NT
100	0	0	NT
10	0	0	NT
Controls			
HPLC H_2O	0	0	0
Vehicle[2]	0	0	0

[1]Formulation is the indicated amount (ppm) of cinnamic aldehyde in a vehicle of 2% Tween 80 and 6% $NaHCO_3$.
[2]Vehicle of 2% Tween 80 and 6% $NaHCO_3$.
[3]Not tested.

Example 5

Effect of Formulation on German Cockroaches

Adult male cockroaches (*Dictyoptera: Blattelidae*) were used to evaluate the insecticidal activity of cinnamic aldehyde and/or coniferyl aldehyde by a topical application method. Twenty cockroaches were placed in stainless steel pails (20 liter) with lids provisioned with food, water and harborage. After one week they were sprayed at arm's length (approximately 1 meter) with 5 ml of a test formulation using a Gilmour spray bottle. The number of dead or moribund cockroaches at 5 minutes, 30 minutes, 1 hour and 12 hours after treatment was counted and compared to those untreated (diluent only). Raid (active ingredients: permethrin, pyrethrins and PBO) was used as a positive control. Within five minutes, all cockroaches treated with 2% cinnamic aldehyde (20,000 ppm) in aqueous vehicle (2% Tween 80, 6% $NaHCO_3$) were dead, as were all those treated with Raid. Ten percent of those treated with vehicle alone were dead in 30 minutes, with no further increase in mortality up to 12 hours.

Example 6

Treatment of Western Subterranean Termites (*Isoptera: Rhinotermitidae*)

Sterilized play sand is treated with aqueous emulsions of each formula and component to provide 500 ppm deposits (wt./wt: sand). 500 g samples of sand are evenly spread $\leq$ 1mm thick over a metal tray (50 by 30) cm). and sprayed with 65 ml of emulsion with an air brush at 1,970 g/cm^2 (28 psi) to obtain uniform treatments. Six examples for each formulae and component are prepared. The treated sand is dried in a fume hood for 30 minutes and the insecticidal activity of each formula treated sand is determined by continuously confining termites to treated deposits for 24 h. Ten termites are exposed on 2.5 ml of treated sand in petri dishes (35 by 10mm) in each of five replicates. Termites and petri dishes are held in a chamber maintained at 93% RH with a saturated sodium sulfate solution. The number of dead or moribund termites after 24 h exposure is determined. Termites are considered dead if unable to right themselves within 5 min. The effectiveness of the test formulation is compared to termites treated with diluent only or with a formulation known to kill termites at a level of about 70%.

Example 7

Effect of Formulation on Ants

The effect of cinnamic aldehyde on adult carpenter ants *(Camponotus pennsylvanicus)* was evaluated as follows. Twenty adult ants were placed in a 20 liter stainless steel pail with lid. The test formulations were prepared and used within one hour and were shaken immediately before spraying the insects. Eight ml of test solution was sprayed with a fine spray (Gilmour hand sprayer). The insects were observed at 0.5, 1, 8 and 24 hours. Cinnamic aldehyde (2%, 20,000 ppm) in 2% Tween 80 and 6% $NaHCO_3$ in water gave 100% mortality at all time points. Raid (active ingredients: permethrin, pyrethrins and PBO) was used as a positive control and gave 90% mortality at 0.5 hr with 100% mortality at all other time points the cinnamic aldehyde at 0.5 hr therefore was superior to Raid.

Example 8

Effect of Formulation on Mosquitos

Adults

The toxicity of the formulation for mosquitos was determined using adult *Aedes aegypti* mosquitos from the University of California Mosquito Control Research Laboratory at the Kearney Agricultural Center. The experiments were performed as double blind studies.

One ml test formulation was pipetted onto 11 cm #2 Whatman filter paper circle cut to fit shell vials (84 mm x 23 mm) which was air dried at room temperature for two hours and placed in a shell vial (84 mm x 23 mm). Twenty unblooded adult female mosquitos approximately four days of age were aspirated using gentle suction into each shell vial. The open end of the vial was covered with 1 mm nylon mesh and filter paper cut to fit for complete coverage from an 11 cm #2 Whatman filter paper circle. The vials were placed in a polyethylene mosquito bag (46 cm x 20 cm) with a wet paper towel inside and loosely sealed. The bag was inflated by gently blowing in air and then placed in an incubator at 22°C for 24 hours with a day light cycle (14 hrs light; 10 hrs dark). Untreated paper and paper treated with H_2O were used as controls. Mortality was determined by counting the number of dead mosquitos.

The efficacy of various concentrations of cinnamic aldehyde in a formulation of 2% Tween 80, 6% $NaHCO_3$ was tested, using concentrations of cinnamic aldehyde ranging from 10 ppm to 25,000 ppm with and without the addition of saponin (1:60 dilution of a 10° Brix solution). At concentrations 100 to 25,000 ppm added to the filter paper, 100% of the mosquitos were killed. At 10 ppm added to the filer paper, 78% of the mosquitos were killed in the absence

of saponin, but only 5% with saponin. 14% of mosquitos were killed with the addition of 2% Tween 80 and 6% $NaHCO_3$ alone (formula blank) to the filter paper and 50% with the further addition of a 1:60 dilution 10° Brix saponin. The percent mortality is the average of three replications, with corrections for control mortality. *See* Table 4. Malathion was used as a positive control.

Table 4

Mosquito Adulticide

Formulation (ppm)	Percent Mortality	
	CNMA	CNMA+SAP
25,000	100	100
12,500	100	100
5,000	100	100
2,500	95	100
100	100	100
10	78	5

% are averages of 3 replications with corrections for control mortality

Control	Percent Mortality
-Control[1]	0
-Control[2]	0
Formula Blank	14
Formula Blank+SAP	50

[1]Plain paper.
[2]H_2O.

Larvae

Larvicidal activity of formulations containing varying concentrations of cinnamic aldehyde were tested in a double blind bioassay on larvae of *Culex quinquefasciatus* mosquito. Twenty-five late 3rd-instar larvae of *Culex quinquefasciatus* were placed in 100 x 80 mm Purex

#3250 glass containers. 250 ml distilled H_2O was pipetted into the containers. One ml of test formulation containing 10 to 25,000 ppm cinnamic aldehyde in vehicle (2% Tween 80 and 6% N_aHCO_3 in distilled H_2O) was pipetted into each container. A control blank using 1 ml distilled H_2O instead of a test formulation also was prepared.

All treated and untreated glass containers were placed in temperature controlled room at 29°C. Each container was evaluated for larvae mortality at 24 hour intervals. The number of dead larvae were reported. *See* Table 5 for results of the bioassay. Concentrations at or above 12,500 ppm cinnamic aldehyde gave 90% mortality at 24 and 48 hours.

Table 5

Larvae

(*Culex quinquefasciatus*)

Treatment (ppm)	Percent Mortality (time)	
	24 hours	48 hours
10	0	0
100	2	2
2,500	4	4
5,000	8	20
12,500	90	90
25,000	100	100
H_2O Control	0	0

Example 9

Treatment of Lice

Determination of Toxicity

Fifty ml of test formula containing various concentrations of cinnamic aldehyde in vehicle (2% Tween 80 and 6% $NaHCO_3$ in distilled H_2O) is applied as evenly as possible to one half of a filter paper disc (5.5 cm in diameter). Two test papers are prepared for each solution. Papers are air dried in a flow of moving air for 30 minutes. Each paper is placed in the center of a 10 cm glass Petri dish. Ten young adult female lice (5-7 hours after engorgement) are placed in the center of the disc and the Petri dish covered. Dishes are placed in an incubator at 30 ± 2°C and approximately 50% humidity.

After 5 minutes, which allows time for the lice to deaggregate and distribute randomly, the lice on the treated side of the filter paper are counted. Dishes are re-examined after each of a further 4 incubation periods of 2 minutes. Any lice found off the filter paper are excluded from the total sample number and are placed back on the filter paper to be counted on the next inspection. Five replications are undertaken on the same day. Scores are summed, as are the total number of lice sampled, and controls checked for random distribution. Toxicity is determined according to the method described in the publication: Standard Test for Effectiveness of Liquid, Gel or Cream Insecticide Against Adult Human Lice (ASTM Designation: E938-83 (re-approved 1988)).

Determination of effect of cinnamic aldehyde on choice of egg-laying site

9 cm diameter filter paper circles are torn into a square and bisected into two triangles by line (pencil). The rough edges of the torn filter paper are attractive egg laying sites. One half of the filter paper is wetted with 200 µl of H_20 or formula and then dried for 30 minutes. A batch of 20 young adult female and 20 young adult male lice are introduced into the petri dish as described above for determination of toxicity and are incubated in the dish at 30 ± 2° C over a 24 hour period. Eggs laid are counted. Tests are repeated over 5 days and egg counts summed for each type of area.

Example 10

Production of aromatic aldehydes in microbial systems

A cDNA library is generated using RNA extracted from six week old tobacco stems. 20µg of polyA RNA is prepared and cDNA synthesized. Part of this is cloned into lambda-ZAP II vector (a commercially available cloning vector). At least 500,000 recombinants are screened using an oligonucleotide probe designed from peptide sequence sequences of CCoAr protein purified from six week old tobacco stem tissue using the protocol of Goffner, et al., *Plant Physiol.* (1994) 106:625. Strongly hybridizing clones are selected and used to rescreen the cDNA library. The resulting clones are sequenced to enable the identification of full-length cDNA inserts and the introduction of appropriate CCoAR gene sequences into yeast expression vector pMTL8110 (Faulkner, et al (1994) *Gene* 143:13-20. The coding sequences for *Rhodosporidium toruloides* phenylalanine ammonia lyase (PAL; GenBank locus RHDPAL) and a parsley 4-coumarate:CoAl ligase (4CL; GenBank locus PC4CL1AA) are similarly introduced into equivalent yeast expression vectors. The PAL,4CL and CCoAR constructs are used to

transform *Saccharomyces cerevisiae* strains by electroporation using established published procedures (Becker, and Guarente, *Methods in Enzymology* 194:182-187, 1991; Simon, (1993) *Methods in Enzymol* 217:478-483. Transformants are selected on minimal medium lacking leucine. Transformant strains carrying all three gene constructs are identified by PCR and selecter for further analysis.

Extracts from both transformed and untransformed control strains are used for determinations of PAL. 4CL and CCoAR enzyme activities using well established published assays. Strains in which the activity of PAL, 4CL and CCoAR is significantly greater than the background activity detected in control strains are selected for further analysis. Selected strains are analyzed for aromatic aldehyde production using standard published procedures and those producing significant amounts of cinnamaldehyde are selected for optimization of fermentation conditions.

Example 11

Treatment of Corn Root Worm with Cinnamic Aldehyde and with Tween 80 and/or $NaHCO_3$

Plant Foliar Bioassay

Plants are grown in 7.5 mm pot in potting soil in greenhouse. Corn plants are used for corn root worm. When plants reach 3 leaf stage, they are infested with 60 of the specified anthropod (6 replications). The corn root worm is allowed to settle and feed. The plant is sprayed to runoff (about 5 ml) with a formulation containing 100 to 2000 ppm, or 0.1 to 2 g/l concentration of a test formulation. The plant is draped with plastic covering to prevent the formulation from touching the soil. The mortality of the worms after three, five and seven days on the plants sprayed with a test formulation is determined and compared with that of worms on plants sprayed only with water and/or a formula blank.

Example 12

Treatment of Russian Wheat Aphid with Cinnamic Aldehyde and with Tween 80 and/or $NaHCO_3$

Plant Foliar Bioassay

Plants are grown in 7.5 mm pot in potting soil in greenhouse. Wheat plants (Kansas variety) are used for Russian wheat aphid. When plants reach 3 leaf stage, they are infested with 60 of the specified anthropod (6 replications). The insect is allowed to settle and fee. The plant

is sprayed to runoff (about 5 ml) with a formulation containing 100 to 10,000 ppm, or 0.1 to 10 g/l concentration of a test formulation. The plant is draped with plastic covering to prevent the formulation from touching the soil. The mortality of the insects after 36 hours, five days and seven days on the plants sprayed with the test formulation is determined and compared with that of insects on plants sprayed only with water and/or a formula blank.

Example 13

Treatment of Thysanoptera with Cinnamic Aldehyde and with Tween 80 and/or $NaCHO_3$

Plant Foliar Bioassay

Plants are grown in 7.5 mm pot in potting soil in greenhouse. Rose plants of various varieties are used for aphids. When plants reach 3 leaf stage, they are infested with 60 of the specified anthropod (6 replications). The insect is allowed to settle and feed. The plant is sprayed to runoff (about 5 ml) with a formulation containing 100 to 10,000 ppm, or 0.1 to 10 g/l concentration of a test formulation. The plant is draped with plastic covering to prevent the formulation from touching the soil. The mortality of the insects after 36 hours, five days and seven days on the plants sprayed with the test formulation is determined and compared with that of insects on plants sprayed only with water and/or a formula blank.

Example 14

Treatment of Melon Aphid

Plant Foliar Bioassay

Treatment of melon aphid (*Aphis gossypii Glover*) was conducted as follows. Plants were grown in 7.5 mm pots in planting soil in a greenhouse. Chrysanthemums (*C.morifolum*) were used for the melon aphid plant foliar bioassays.

Treatment of flowering plants with cinnamaldehyde

Flowering chrysanthemum plants were infested and pre-count population size for each plant were taken and number of mean of aphids nymphs per leaf calculated. The plants were sprayed to runoff (about 5 ml) with an aqueous formulation containing 1,000 ppm, 3,000 ppm, and 10,000 ppm concentration of cinnamic aldehyde, or a negative control containing only H_2O. After 36 hours, the number of insects on the leaves sprayed with a given test formulation was determined and compared with that of insects on leaves sprayed with the negative control only.

The number of mean aphid nymphs per leaf was determined to be less than 10 for each cinnamic aldehyde concentration as compared to a pre-count mean of about 60 and to the negative control of about 33. *See* Table 6.

Table 6

Melon Aphid

Formulation	Mean Number of Aphid Nymphs Per Leaf
CNMA (ppm)*	
1,000	6 ± 3
3,000	4 ± 3
10,000	1 ± 1
Control	
H_2O	33 ± 11
Pre-count	60

*CNMA = cinnamic aldehyde (ppm) in H_2O.

Treatment of plants with cinnamaldehyde and saponin

Whole nonflowering potted chrysanthemum plants were infested and were used to assay the effect of cinnamic aldehyde and saponin on melon aphids. Two plants were treated for each concentration and two leaves, one from the top of the plant and one from the bottom of the plant, were sampled to determine the number of living and dead melon aphids. One of three treatments was applied: 1.0% CNMA plus 0.5% Saponin, 0.5% CNMA plus 0.25% Saponin, and 0.5% Saponin only. The whole plant was sprayed to "drip" on both the top and bottom sides of the leaves. Results are presented as the proportion of aphids found dead of the total number of aphids on the plants. The results were as follows: control plant (0.5% Saponin only) 14.8% ± 4.5; 0.5% CNMA 48.3 ± 16.1; 1.0% CNMA 72.0 ± 11.2. These results indicate that the CNMA alone or with saponin can kill a high percentage of melon aphids following direct application to the plant.

Example 15

Treatment of Spiders

Contact treatment

To determine the contact activity of the formulae, test arachnids *Latrodectus spp* and *Laxosceles reclusa* are directly sprayed. The treated spiders are carefully removed and placed in untreated petri dishes or vials. Five different concentrations of each active ingredient in a formula are directly sprayed onto the test spider. A formula blank and a negative control are tested. Five replicates are tested with each formula and spider. Mean mortality of spiders are determined for each treatment at 24 and 48 hours.

Example 16

Treatment of Scab Mite

Contact treatment

Scab Mite (or Psoroptic Scab)(*Psoroptes equi* (Raispail) and *P. ovis* (Hering)) are tested to determine the contact insecticidal activity of the subject formulae. Test mites are directly sprayed with a given test formula. Treated mites are removed and placed in untreated petri dishes or vials. Five different concentrations of each active ingredient in a formula are directly sprayed onto the test scab mite. A formula blank and a negative control also are tested. Five replicates are tested for each formula. Mean mortality of mites are determined at 24 and 48 hours for each treatment.

Example 17

Treatment of Bed Bug

To determine the contact activity of the cinnamaldehyde (CNMA) and alpha hexyl cinnamaldehyde (HCA) formulae, test bed bugs (*Cimex lectularis*) are directly sprayed with a given test formulation. The treated bed bugs are removed and placed in untreated petri dishes or vials. Five different concentrations of each active ingredient in a formula are directly sprayed onto the test bed bug. As a control, a formula blank and a negative control (H_20) are tested. Mean mortality of bed bugs are counted at 24 and 48 hours for each treatment.

Example 18

Residual Activity of Cinnamaldehyde and α-hexyl Cinnamaldehyde

Two separate experiments indicated that both cinnamaldehyde (CNMA) and alpha hexyl cinnamaldehyde (HCA) have residual activity. In the first experiment, two ml of two concentrations of CNMA (0.3 and 1%) were sprayed on filter paper (Whatman). As a negative control, two ml of water was also sprayed on filter paper. Twenty-four hours later, two ml of water were sprayed on treatment and control filter paper, which were then dried for 30 min. Approximately 30 thrips insects (*Frankliniella occidentalis*) were introduced onto the treated filter papers and the number of *F. occidentalis* were observed after one hour. Mean mortality was calculated for each treatment. After 72 hours, the treated filter papers were flipped over and only the negative control filter paper and the filter paper treated with 1% CNMA were sprayed with 2 ml of water and allowed to dry for 30 minutes. Approximately 30 thrips were introduced onto the two treated filter papers and after one hour the number of dead *F. occidentalis* were observed and the mean mortality calculated for each treatment. A similar assay was conducted using HCA. Mean mortality was higher for rehydrated filter papers compared to non-rehydrated filter papers over time. These experiments demonstrate that rehydration plays a role in the continued lethal effects of treated filter paper in contact with thrips.

To further determine the residual activity of CNMA and HCA, insects are confined to deposits on two representative surfaces. Glass is used to represent non-porous surfaces and filter paper is used as a porous surface. Two ml of five different concentrations of each active ingredient in a formula are applied to filter paper disks (9 cm diameter) or the bottoms of glass petri dishes (9 cm diameter). As a control, two ml of formula minus active ingredient are also applied. The deposits are allowed to dry for 24 hours before testing. At test intervals of 7, 14, 28, and 56 days. one set of plates and filter papers are rehydrated with 2 ml of water, while a parallel set is not rehydrated. Insects are then confined to the deposits continuously and the number of insects killed by the deposits is counted regularly. If deposits fail to kill insects within 48 hours, these treatments are discontinued from further aging studies.

Example 19

Control of Mealybugs

To determine the contact activity of the cinnamaldehyde (CNMA) and alpha hexyl cinnamaldehyde (HCA) formulae. test mealybugs are sprayed directly with a given test formulae.

The treated insects are removed and placed in sterile untreated petri dishes or vials. Five different concentrations of each active ingredient in a formula are directly sprayed onto the test mealybug. As a control, a formula blank and a negative control (H_20) are tested. Five replicates are tested with each formula. Mean mortality of mealybugs are determined for each treatment at 24 and 48 hours.

Example 20

Evaluation of Storax Combined with Cinnamic Aldehyde or α-Hexyl Cinnamic Aldehyde Against Melon Aphid

In previous bioassays evaluating the efficacy of cinnamic aldehyde against melon aphid (*Aphis gossypli Glover*) on plants sprayed to run-off with an aqueous formulation of cinnamic aldehyde at 1.000 ppm, an LD_{50} was observed at 2 hrs and an LD_{75} at 24 hrs. The purpose of this study was to determine the effect of STORAX incorporated into various formulations, with and without cinnamic aldehyde and α-hexyl cinnamic aldehyde, to evaluate its potential use as a synergist. Initial bioassays were conducted using STORAX at 0.6%, 1.0%, 2.0% with 1% Tween 80 in water. The results are presented in Figure 1. Another bioassay was conducted to evaluate the efficacy of STORAX (at 0.6%) alone with 1% Tween 80 in water and STORAX (at 0.6%) plus cinnamic aldehyde (at 0.1%) or alpha hexyl cinnamaldehyde (at 0.1%) with 1% Tween 80 in water. The results are presented in Figure 2. STORAX combined with cinnamic aldehyde or α-hexyl cinnamic aldehyde reduces the time course of lethality and increases the mortality. Moreover, the cinnamic aldehyde-STORAX formulation approaches the lethal time (LT) required at 2h for kill of certain virus transmitting pests (e.g., brown citrus aphid). Observations indicate that STORAX inhibits phytotoxicity for foliar application at an aldehyde concentration <0.5% on sensitive plants (e.g., glasshouse rose varieties).

Example 21

Proposed Bioassay of Pesticide Efficacy Against Brown Aphid

Preliminary bioassays using the formulations listed below have shown a high degree of efficacy against aphid populations such as the Melon aphid. The results thus far show that these materials can kill a high percentage of the aphid population in a relatively short time period (up to 95% in <3 hr at some concentrations). The following protocol was designed to evaluate the efficacy at the indicated formulations against the brown aphid and to estimate the lethal dosage

(LD) and lethal time (LT) of the different treatment regimens on the brown aphid. The brown aphid infects citrus trees with the potent virus called tristeza. To be effective in the field, a significant degree of lethality (LD90+) is required within 2 h of treatment. The treatment regimen and percent by weight of the test compound are shown in the following Table.

Table 7

Treatment	CNMA or HCA Levels (% by weight)
Water + Tween 80 (1.0%) only	-----
Storax (1%) + Tween 80 (1.0%) only	-----
CNMA + Tween 80 (1.0%)	0.1, 0.25, 0.50
Storax (1.0%) + CNMA + Tween 80 (1.0%)	0.1, 0.25, 0.50
Storax (1.0%) + HCA + Tween 80 (1.0%)	0.1, 0.25, 0.50
Water only	-----

Trials are conducted using ≥ 4 replicates per treatment and approximately 50 or more aphids per replicate. This results in a total of 44 observations for the trial. Material is applied by foliar spray to run off on each plant at the prescribed concentrations by volume as presented in the treatment list above. The number of aphids killed for each treatment is recorded at 1 h, 2 h, 6 h, and 24 h. The LD is calculated from the total proportion of aphids killed for a given dosage of active ingredient in the formulations. LT is calculated by determining the elapsed time to reach a proportion killed at a given formula concentration.

Example 22

Bioassay of Pesticide Efficacy and Phytotoxicity

Preliminary bioassays using the active ingredients and formulations listed in the Table below have shown no observable phytotoxicity on subject plants. The following protocol is designed as a preliminary evaluation of the phytotoxicity of the indicated formulations on roses.

Table 8

Treatment	CNMA or HCA Levels (% by weight)
Water + Tween 80 (1.)%) only	------
Storax (1.0) + Tween 80 (1.)%) only	------
CNMA + Tween 80 (1.0%)	0.1, 0.25,0.50
Storax (1.0%) + CNMA + Tween 80 (1.0%)	0.1, 025, 0.50.
Storax (1.0%) + HCA+ Tween 80 (1.0%)	0.1, 0.25, 0.50
Water only	------

Phytotoxicity trials are conducted using a 4 by 3 design (4 repetitions with three observations per repetition per treatment). Tests compare the effect of 12 formulation treatments, 9 containing one or more active ingredients and 3 control treatments for comparison with respect to phytotoxicity symptoms. Materials are applied with a hydraulic sprayer to drip. Up to 3 treatment applications are made at 7 day intervals for each formulation. Phytotoxicity symptoms are assessed visually at 3 days post-application for each of the three applications.

Example 23

Treatment of Slugs (*Milax gagates (Draparnaud)* and Snails (*Helix aspersa Miller*) with cinnamic aldehyde encapsulated bait.

Cinnamic aldehyde (2%) was sub-microencapsulated at the one micron size in a beeswax solution (prepared by Sun Smart, Long Island, NJ). A sub-microencapsulated formula blank, at the same one micron size was prepared using beeswax only. Oatmeal (Quaker Oats) was used as a bait substrate. Approximately 0.5 gram of oatmeal was mixed with 2 ml of encapsulated cinnamic aldehyde and placed in a 1-1/2" diameter culture dish (Carolina Biologic P7-74-1996). Four such culture dishes were prepared as bait sources and one dish placed in one of four terraria bioassay arenas. Each terrarium, complete with dial-ventilated top, measures 11" x 7" x 8" high including the top and is obtained from Connecticut Valley Biologic A2109). Two similar culture bait source dishes were prepared using the same weight of oatmeal but with 2 mls of encapsulated beeswax only (formula blank). The formula blank bait sources were each placed in one of two terraria bioassay arenas as described above. Eleven slugs or snails were introduced into each arena. A 5 cm x 5 cm x 1 cm sponge moistened with 2 mls of H_2O was placed in each arena. Mortality was observed at 72 hours (*see* Table 9).

Table 9

Target	Terraria	Mortality - 72h		Bait
		Alive	Dead	consumed %
Snails *Helix aspersa*	Red #1 Encapsulated 2% CNMA	6*	5	5%
	Red #2 Encapsulated 2% CNMA	7*	4	2%
	Yellow #1 Encapsulated Formula Blank	10	0	100%
Slugs *Milax gagates*	Red #3 Encapsulated 2% CNMA	0	11	5%
	Red #4 Encapsulated 2% CNMA	0	11	6%
	Yellow #2 Encapsulated Formula Blank	0	1	40%

* refused to approach bait.

Example 24

Treatment of German cockroach (*Blatella germanica (Linnaeus)*) with cinnamic aldehyde encapsulated bait.

Cinnamic aldehyde (2%) was sub-microencapsulated at the one micron size in a beeswax solution (*see* Example 23). A sub-microencapsulated formula blank, at the same one micron size was prepared using beeswax only. Oatmeal (Quaker Oats) was used as a bait substrate. Approximately ½ gram of oatmeal was mixed with 2 ml of encapsulated cinnamic aldehyde and placed in a 1-1/2" diameter culture dish (Carolina Biologic P7-74-1996). Two such culture dishes were prepared as bait sources and one dish placed in one of two terraria bioassay arenas as described in Example 23. Two similar culture bait source dishes were prepared using the same weight of oatmeal but mixed with 2 mls of encapsulated beeswax only (formula blank). The formula blank bait sources were each placed in one of two terraria bioassay arenas as described above. Nine German cockroaches were introduced into each arena. A 5 cm x 5 cm x 1 cm sponge moistened with 2 mls of H_2O was placed in each arena. Mortality was observed at 72 hours (*see* Table 10).

Table 10

Target	Terraria	Mortality - 72h		Bait
		Alive	Dead	consumed %
German cockroaches	Red #1 Encapsulated 2% CNMA	4*	5	3%
	Red #2 Encapsulated 2% CNMA	2*	7	4%
	Yellow #1 Encapsulated Formula Blank	7	2	30%
	Yellow #2 Encapsulated Formula Blank	7	2	25%

* refused to approach bait.

Example 25

Treatment of Melon Aphid on Chrysanthemum with Cinnamic Aldehyde encapsulated in two different wax shells (Beeswax and Carnauba wax)

Cinnamic aldehyde (1%) was sub-microencapsulated at the one micron size in beeswax or carnauba wax solution (prepared by Sun Smart, Long Island, NJ). Treatment of melon aphid (*Aphis gossypii Glover*) was conducted as follows. Chrysanthemum (*C. morifolium*) leaves infested with melon aphid were selected at random and assigned to either a treatment (T1 or T2) or control block (C1 or C2). A total of 9 leaves, each leaf a replicate, was sealed in a carton and assigned to each treatment or control. Using a laboratory spray tower calibrated to spray the field equivalent of 113 liter per acre at the desired dosage, T1 and T2 and their control formula blanks, C1 and C2, were sprayed. For each treatment, cartons received treatment and were maintained at room temperature. The mortality of melon aphid was observed for each spray at 24 hours and the percent mortality recorded (*see* Table 11).

Table 11

Encapsulated	Melon Aphid (*Aphis gossypii Glover*) Mortality at 24 hrs.
T1 (1% Cinnamic Aldehyde in carnauba wax shell)	90%+
T2 (1% Cinnamic Aldehyde in beeswax shell)	90%+
C1 Formula blank - carnauba	5%
C2 Formula blank - beeswax	5%

Example 26

Treatment of 1st and 2nd Instars of Silverleaf Whitefly (*Bemisia argentifolii*) with Cinnamic aldehyde on Poinsettia (*Euphorbia pulcherrima*)

Clip cages were set on 3 month old poinsettia plants leaves so that white fly adults were captured within the cages. The white flies were allowed to oviposit for 48 hours. Four plants per treatment or control were put into an environmental chamber and allowed to incubate at room temperature for 5 days until the majority of eggs had hatched to first or second instar. The first and second instar were selected because they were known to be more resistant to pesticides. Three treatments at 0.5% cinnamic aldehyde and 0.25% Tween 20 (T1, T2, T3) and three control treatments of 0.25% Tween 20 only (C1, C2, C3) were sprayed to run off on each plant assigned to treatment. Mortality of the instars was recorded after 48 hours (*see* Table 12)

Table 12

	Silverleaf Whitefly Instars (1 and 2) Dead	Alive	% Mortality
T1	5		
T2	13		
T3	17		
	35		91%
C1	33		
C2	220		
C3	262		
	515		9%

Example 27

Treatment of Mites on Bean Leaf Disk.

α-hexylcinnamic aldehyde (HCA) at a concentration of 0.1, 0.3 or 1.0% was submicroencapsulated at the one micron size in beeswax or carnauba wax. The microencapsulated α-hexyl cinnamic aldehyde was prepared by Sun Smart (Long Island, NJ). Leaf disks (20 mm diameter) were cut from bean leaves and placed on moist cotton. Then ten adult female mites were placed on each leaf disk and sprayed in the Potter spray tower with 2 ml of one of the test concentrations; control mites were treated with distilled water. A total of 30 mites (3 leaf disks) were treated with each concentration. Sprayed leaf disks were held at high humidity at 70°F in a growth chamber. Mortality was assessed 48 hours and 72 hours after the mites were sprayed. Results are summarized in Table 13.

Table 13

Treatment	Concentration %	No. Mites Treated	% Mortality after 48 hr.	% Mortality after 72hr
HCA in carnauba wax	0.1	45	11.1	17.8
	0.3	55	41.8	52.7
	1.0	55	100.0	100.0
Untreated control		55	12.8	12.8
HCA in beeswax	0.1	30	16.7	36.7
	0.3	30	27.5	40.0
	1.0	30	97.5	100.0

Both the carnauba wax and the beeswax formulations containing HCA appeared to be equally effective. For both formulations, the maximum effective dose was between 0.1 and 1.0%; intermediate concentrations between 1.0 and 0.3% need to be evaluated to better define the dose response lines. All mites treated with the 1.0% concentration died after 72 hours. Both formulations left a noticeable white residue on the leaf surface.

Example 28

Treatment of Melon aphid on Chrysanthemum
with 5% alpha Hexylcinnamic aldehyde
in emulsion and microencapsulated

Treatment of melon aphid (*Aphis gossypii* Glover) on chrysanthemum leaves was conducted to evaluate the efficacy of α-hexylcinnamic aldehyde (HCA) in different formulations. Efficacy was compared among three treatments (5% HCA, 1% Tween 20 in water; 5% HCA microencapsulated in beeswax; 5% HCA microencapsulated in carnauba wax; and a water only control. Three chrysanthemum leaves infested with melon aphids were used for each treatment. Four replicates (4 observations per treatment) were compared for each formulation. Tests were conducted using 500 ml ventilated paper cartons as experimental arenas containing approximately 50 adult *Aphis gossypii* in each per leaf (150 aphids per arena). For each test, treatment cartons received HCA in a 5% aqueous emulsion or microencapsulated in either beeswax or carnauba wax. The negative control was a water only treatment. Sprays of each treatment were applied to cartons using a laboratory spray tower calibrated to spray the field equivalent of 113 liters per acre. The paper carton arenas were maintained at room temperature. Experimental cartons were examined at 24 hours post-treatment and % mortality of the melon aphids determined (*see* Fig. 3).

The above results demonstrate that formulations containing aromatic aldehydes, as exemplified by cinnamic aldehyde and α-hexyl cinnamic aldehyde, are effective in killing pests including disease carrying insects, insects and arachnids.

The experiments demonstrate that the formulations are effective in treating roses for powdery mildew, rust, and aphid infestations and grapes for phylloxera infestation with the formulations being effective at the egg, nymphal, and adult life stages of phylloxera. The formulations also were shown to be effective against pests such as spider mite, aphid, white fly, nematode, snails, slugs, cockroaches and thrips. The formulations also were effective against fungal pathogens such as *Fusarium subglutinans*, *Botrytis cinera*, and turfgrass pathogens such as *Sclerotinia* dollar spot, *Rhizoctonia* blight and *Pythium* blight.

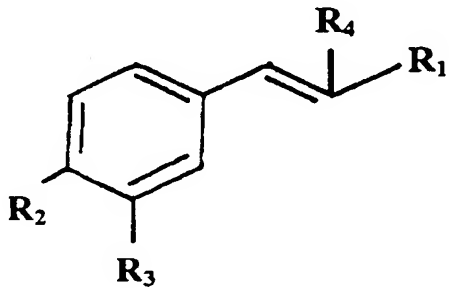
All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now having been fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. A composition comprising microcapsules at a size about 0.1 to 50 micron, said microcapsules comprising a polymer shell which encapsulates a formulation comprising an effective pest or arachnid inhibiting amount of an aromatic aldehyde having the formula



(2)

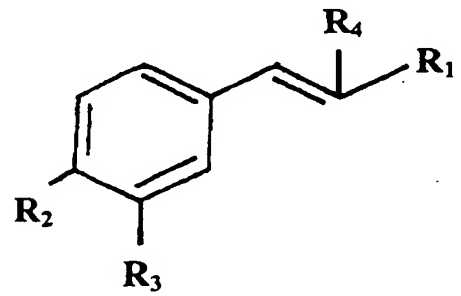
wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms.

2. The composition according to Claim 1, wherein said aromatic aldehyde is the method according to Claim 16, wherein said aromatic aldehyde is cinnamic aldehyde, α-hexyl cinnamic aldehyde and coniferyl aldehyde.

3. The composition according to Claim 1 or 2, wherein said polymer is beeswax or carnauba wax.

4. The composition according to any of the Claims 1-3, wherein said effective pest or arachnid amount is 0.1-10%.

5. A composition comprising a balsam and an aromatic aldehyde having the formula



(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1

to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms.

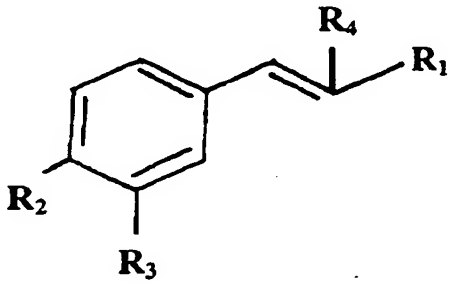
6. The composition according to Claim 5, wherein said balsam is derived from a *Liquidambar* tree.

7. The composition according to Claim 6, wherein said *Liquidambar* tree is *Liquidambar orientalis Miller* or *Liquidambar sytraciflua.*

8. The composition according to Claim 5, wherein said aromatic aldehyde is cinnamic aldehyde, alpha-hexyl cinnamic aldehyde or coniferyl aldehyde.

9. A method for inhibiting infestation of insects or arachnids of a plant part or a plant surface, said method comprising:

contacting said plant part or plant surface with a nonphytotoxic composition comprising a balsam and an aromatic aldehyde having the formula



(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms.

10. The method according to Claim 9, wherein said composition further comprises a surfactant.

11. The method according to Claim 9 or 10, wherein said composition further comprises at least one of cinnamic aldehyde, alpha-hexyl cinnamic aldehyde and coniferyl aldehyde.

12. The method according to Claim 9, wherein said insect is an aphid.

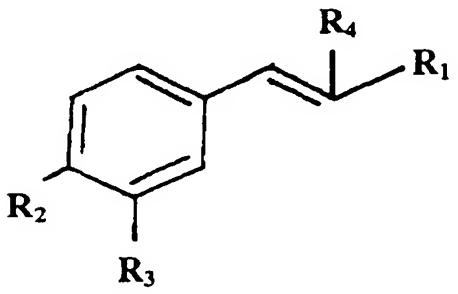
13. The method according to Claim 12, wherein said aphid is a melon aphid or a brown aphid.

14. The method according to Claim 9, wherein said plant surface or plant part is cotton plant surface or part.

15. The method according to Claim 9, wherein said plant surface or plant part is a citrus plant surface or part.

16. A method for controlling growth of an insect or arachnid population, said method comprising:

contacting said insect or arachnid with a composition comprising microcapsules which encapsulate an effective insect or arachnid growth inhibiting amount of an aromatic aldehyde having the formula



(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms.

17. The method according to Claim 16, wherein said aromatic aldehyde is cinnamic aldehyde, alpha-hexyl cinnamic aldehyde and coniferyl aldehyde.

Figure 1

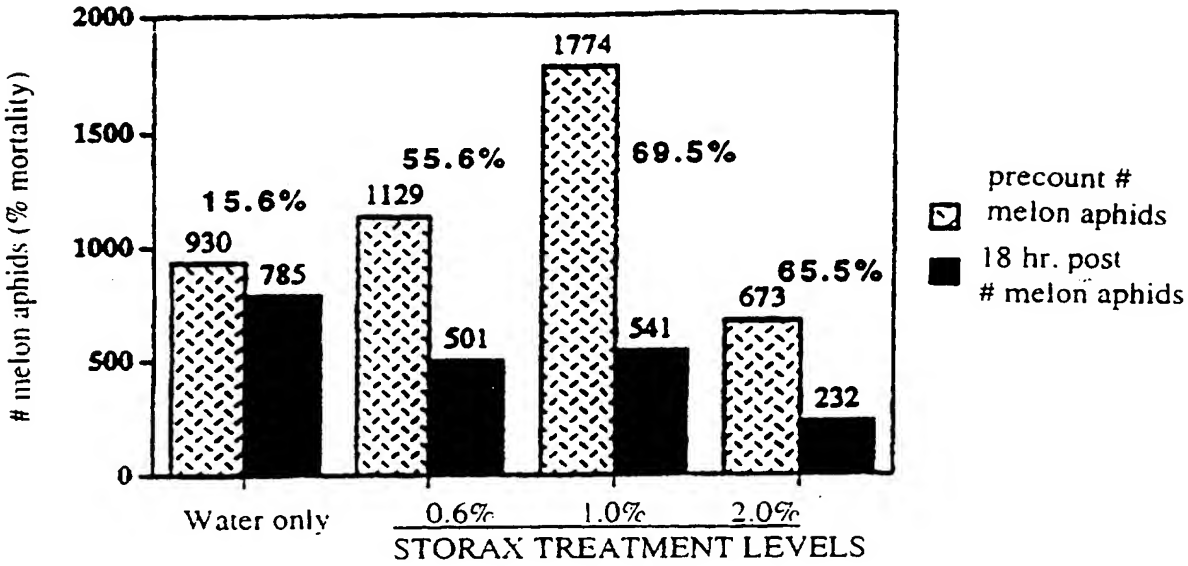
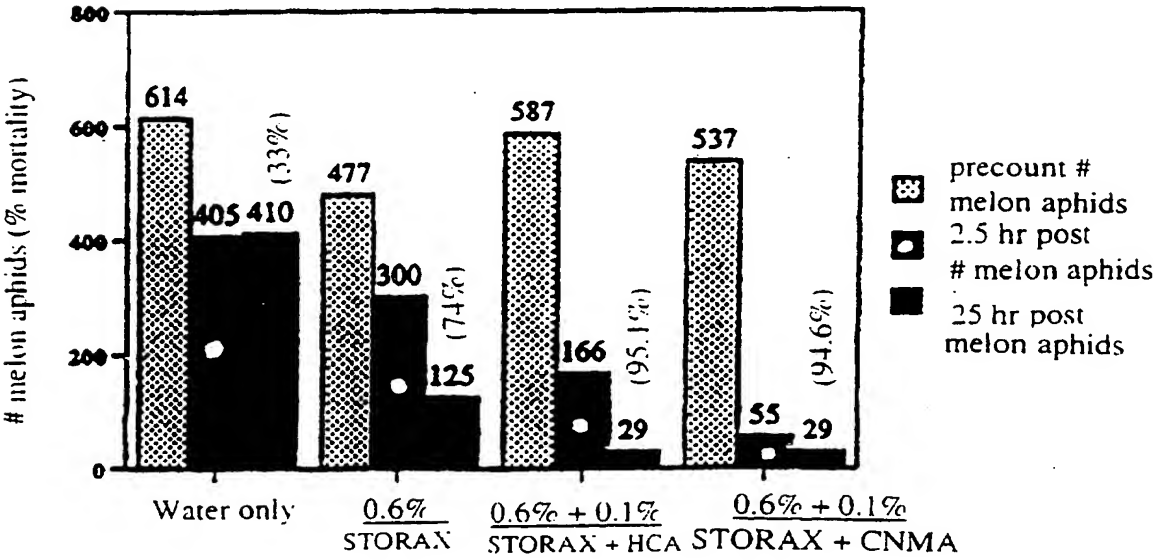


Figure 2



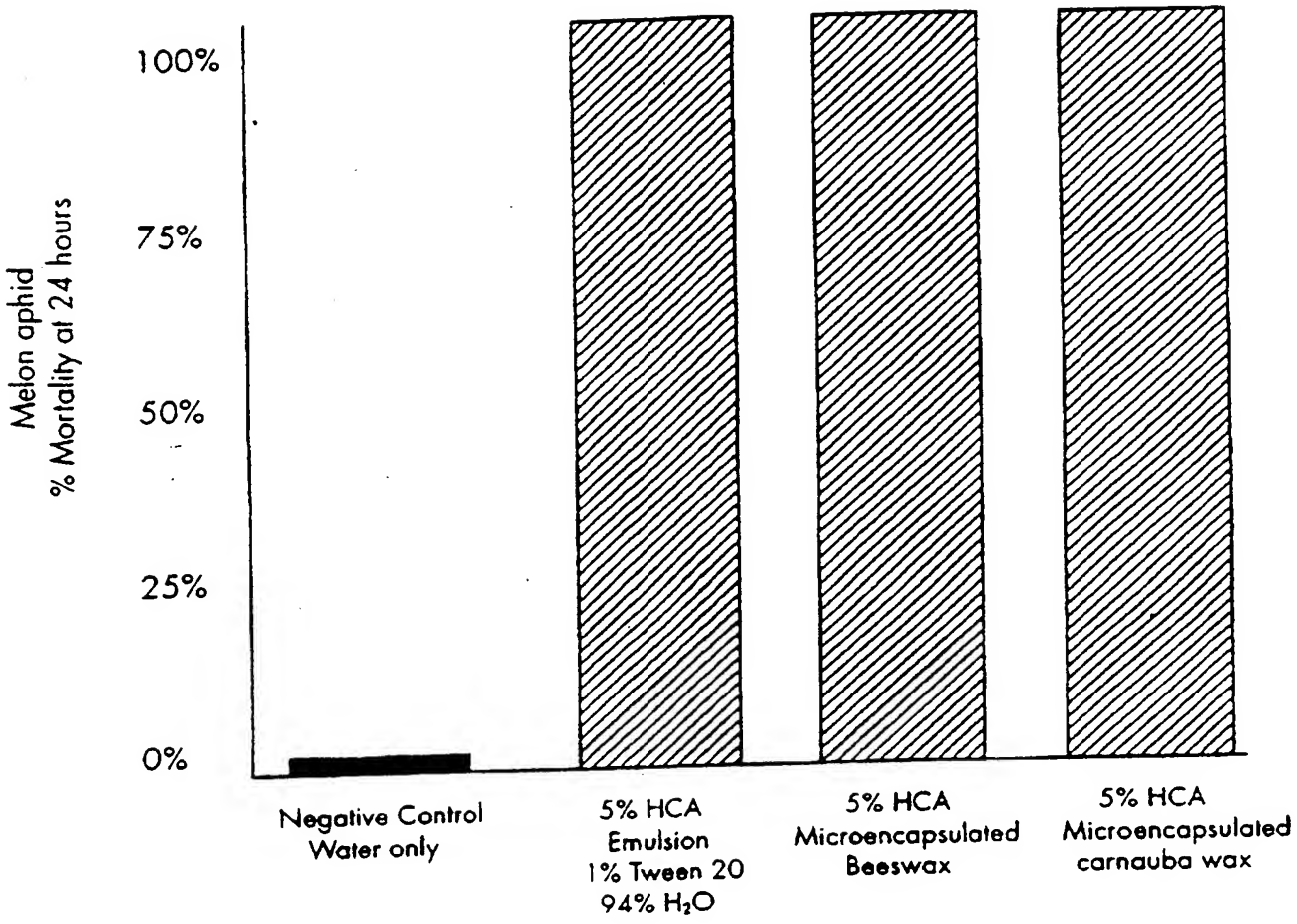

Melon aphid
% Mortality at 24 hours
100%
75%
50%
25%
0%
Negative Control
Water only
5% HCA
Emulsion
1% Tween 20
94% H_2O
5% HCA
Microencapsulated
Beeswax
5% HCA
Microencapsulated
carnauba wax

Figure 3

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(54) Title: USE OF AROMATIC ALDEHYDES AS INSECTICIDES AND FOR KILLING ARACHNIDS

(57) Abstract

Methods and compositions based upon natural aromatic compounds are provided, which find use as pesticides. The pesticides are formulated in a variety of ways, including dusts, sprays, shampoos, soaps and microcapsules, and can be bound to a solid support or provided as bait or directly impregnated into organic matter infested by or susceptible to infestation by a target pest. Pests controlled include aphids mosquitos, lice, ants, snails, slugs, cockroaches, lice, and ticks.

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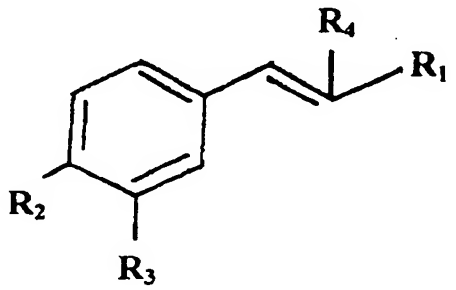
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

AMENDED CLAIMS

[received by the International Bureau on 17 November 1997 (17.11.97);
original claims 1, 2, 4, 5 and 16 amended; remaining claims unchanged (3 pages)]

1. A composition comprising microcapsules at a size about 0.1 to 50 micron. said microcapsules comprising a polymer shell which encapsulates a formulation comprising an effective pest or arachnid inhibiting amount of an aromatic aldehyde having the formula



(2)

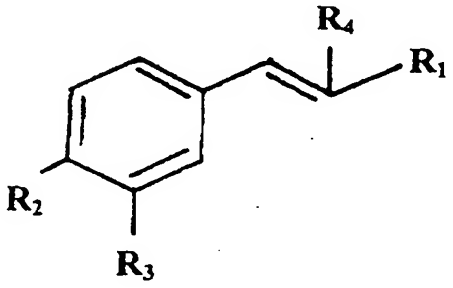
wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms, wherein said effective pest or inhibiting amount is 0.1 to 20% by weight of said microcapsules.

2. The composition according to Claim 1, wherein said aromatic aldehyde is cinnamic aldehyde, α-hexyl cinnamic aldehyde or coniferyl aldehyde.

3. The composition according to Claim 1 or 2, wherein said polymer is beeswax or carnauba wax.

4. The composition according to any of the Claims 1-3, wherein said effective pest or arachnid inhibiting amount is 0.1-10%.

5. A composition comprising a balsam and an aromatic aldehyde having the formula



(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms, wherein said balsam is 0.1 to 2% by weight of said composition.

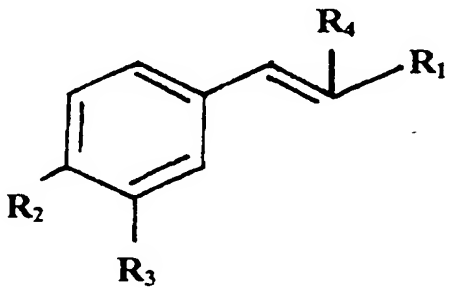
6. The composition according to Claim 5, wherein said balsam is derived from a *Liquidambar* tree.

7. The composition according to Claim 6, wherein said *Liquidambar* tree is *Liquidambar orientalis Miller* or *Liquidambar sytraciflua.*

8. The composition according to Claim 5, wherein said aromatic aldehyde is cinnamic aldehyde, alpha-hexyl cinnamic aldehyde or coniferyl aldehyde.

9. A method for inhibiting infestation of insects or arachnids of a plant part or a plant surface, said method comprising:

contacting said plant part or plant surface with a nonphytotoxic composition comprising a balsam and an aromatic aldehyde having the formula



(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms.

10. The method according to Claim 9, wherein said composition further comprises a surfactant.

11. The method according to Claim 9 or 10, wherein said composition further comprises at least one of cinnamic aldehyde, alpha-hexyl cinnamic aldehyde and coniferyl aldehyde.

12. The method according to Claim 9, wherein said insect is an aphid.

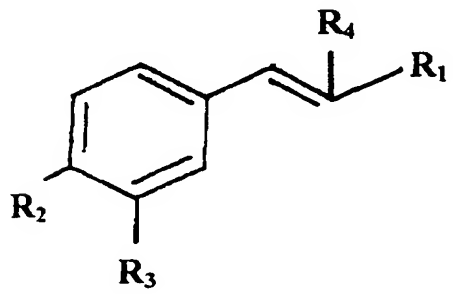
13. The method according to Claim 12, wherein said aphid is a melon aphid or a brown aphid.

14. The method according to Claim 9, wherein said plant surface or plant part is cotton plant surface or part.

15. The method according to Claim 9, wherein said plant surface or plant part is a citrus plant surface or part.

16. A method for controlling growth of an insect or arachnid population, said method comprising:

contacting said insect or arachnid population with a composition comprising microcapsules at a size of about 0.1 to 50 micron, which encapsulate an effective insect or arachnid growth inhibiting amount of an aromatic aldehyde having the formula



(2)

wherein R_1 represents-CHO, R_2 represents -H, -OH or an organic substituent containing from 1 to 10 carbon atoms, R_3 represents -H, a methoxy group, or organic substituent containing from 1 to 10 carbon atoms, and R_4 represents a hydrogen or an organic substituent containing from 1 to 10 carbon atoms.

17. The method according to Claim 16, wherein said aromatic aldehyde is cinnamic aldehyde, alpha-hexyl cinnamic aldehyde and coniferyl aldehyde.

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(57) Abstract

Methods and compositions based upon natural aromatic compounds are provided, which find use as pesticides. The pesticides are formulated in a variety of ways, including dusts, sprays, shampoos, soaps and microcapsules, and can be bound to a solid support or provided as bait or directly impregnated into organic matter infested by or susceptible to infestation by a target pest. Pests controlled include aphids mosquitos, lice, ants, snails, slugs, cockroaches, lice, and ticks.

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INTERNATIONAL SEARCH REPORT

Interr ›nal Application No
PCT/US 97/05369

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N35/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 October 1997	20.10.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fort, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 118, no. 11, 15 March 1993 Columbus, Ohio, US; abstract no. 95878, YUAN ET AL.: "Application of microencapsulation for toxicology studies. III Bioavaibility of microencapsulated cinnamaldehyde" XP002034800 see abstract & FUNDAM. APPL. TOXICOL., vol. 20, no. 1, 1993, pages 83-87, ---	1-4,16, 17
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Y	FR 2 529 755 A (SAOTOME) 13 January 1984 cited in the application *see the whole document* ---	5-15
Y	US 4 978 686 A (KIYOSHI SOTOME) 18 December 1990 cited in the application *see the whole document* ---	5-15
Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 82-73641E XP002034801 see abstract & JP 57 120 501 A ---	5-15
Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 92-223400 XP002034802 see abstract & JP 04 149 103 A ---	5-15
Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 85-027823 XP002034803 see abstract & JP 59 222 402 A ---	5-15

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/05369

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

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Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/US 97/05369

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